



(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
28.07.2021 Bulletin 2021/30

(51) Int Cl.:
G01N 33/574 (2006.01)

(21) Application number: **20211725.5**

(22) Date of filing: **19.03.2013**

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

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(30) Priority: **19.03.2012 US 201261612826 P**

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(62) Document number(s) of the earlier application(s) in accordance with Art. 76 EPC:
18189485.8 / 3 431 997
13763504.1 / 2 828 664

Remarks:

- This application was filed on 03-12-2020 as a divisional application to the application mentioned under INID code 62.
- Claims filed after the date of receipt of the divisional application (Rule 68(4) EPC).

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(54) **METHODS FOR TREATING AND MONITORING THE STATUS OF CANCER**

(57) Provided herein are methods for treating cancer in a subject comprising administering to the subject a therapeutically effective amount of a peptide derived from the EphA2 protein and/or the IL-13R α 2 protein and monitoring the amount of cancer stem cells in said subject. Also provided herein are methods for monitoring the ef-

ficacy of an EphA2 peptide-based cancer treatment or an IL-13R α 2 peptide-based cancer treatment in a patient with cancer, comprising monitoring the amount of cancer stem cells in said subject prior to, during, and/or following cancer treatment of a patient.

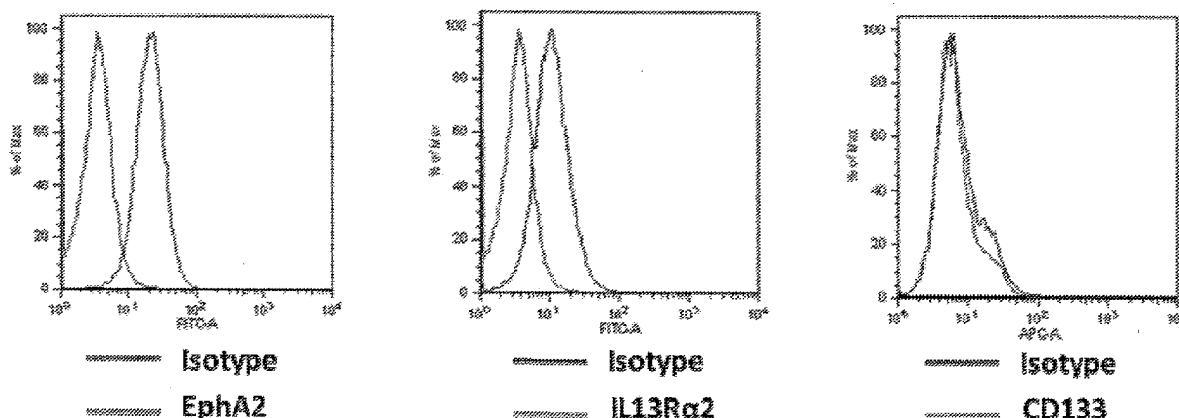


FIG. 1

Description**1. INTRODUCTION**

[0001] Provided herein are methods for treating cancer in a subject comprising administering to the subject a therapeutically effective amount of a peptide derived from the EphA2 protein or the IL-13R α 2 protein and monitoring the amount of cancer stem cells in said subject. Also provided herein are methods for monitoring the efficacy of an EphA2 peptide-based cancer treatment or an IL-13R α 2 peptide-based cancer treatment in a patient with cancer, comprising monitoring the amount of cancer stem cells in said subject prior to, during, and/or following cancer treatment of a patient.

2. BACKGROUND

[0002] Conventional cancer therapies include surgery, chemotherapy, and radiation therapy. Despite the existence of these therapies, as well as the significant amount of scientific and medical research dedicated annually to uncovering cancer therapeutics, cancer remains one of the leading causes of mortality and morbidity worldwide today. As such, there remains a need for new and effective cancer therapeutics, as well as methods for monitoring the efficacy of existing and new cancer therapeutics.

3. SUMMARY

[0003] In one aspect, provided herein are methods of treating cancer in a subject, comprising administering to said subject a therapeutically effective amount of a peptide derived from the EphA2 protein and monitoring the amount of cancer stem cells in said subject. In a specific embodiment, the amount of cancer stem cells in said subject that express EphA2 is measured. In another specific embodiment, the amount of cancer stem cells in said subject that express CD133 is measured. In another specific embodiment, the amount of cancer stem cells in said subject that express EphA2 and CD 133 is measured.

[0004] In another aspect, provided herein are methods for monitoring the efficacy of an EphA2 peptide-based cancer treatment (i.e., a treatment or therapy that comprises administration of a peptide derived from EphA2) for a patient with cancer, comprising monitoring the amount of cancer stem cells in said subject prior to, during, and/or following the cancer treatment of a patient. In a specific embodiment, the amount of cancer stem cells in said subject that express EphA2 is measured. In another specific embodiment, the amount of cancer stem cells in said subject that express CD133 is measured. In another specific embodiment, the amount of cancer stem cells in said subject that express EphA2 and CD133 is measured.

[0005] In another aspect, provided herein are methods for treating cancer comprising administering a T cell epitope of EphA2 that targets cancer stem cells, wherein said epitope is sufficient to induce an immune response in a patient with cancer.

[0006] In another aspect, provided herein are methods of treating cancer in a subject, comprising administering to said subject a therapeutically effective amount of a compound that targets the EphA2 protein, wherein said compound is capable of killing and/or preventing the differentiation of cancer stem cells that express the EphA2 protein. In a specific embodiment, the compound is an antibody that specifically binds EphA2.

[0007] In another aspect, provided herein are methods of improving the targeting of cancer stem cells with a cancer vaccine comprising determining the binding motif of a Class I or Class II epitope from EphA2, and making substitutions in the amino acid sequence such that the modified peptides are able to induce an immune response that is at least as effective at killing cancer stem cells as the wild type peptide.

[0008] In another aspect, provided herein are methods of treating cancer in a subject, comprising administering to said subject a therapeutically effective amount of a peptide derived from the IL-13R α 2 protein and monitoring the amount of cancer stem cells in said subject. In a specific embodiment, the amount of cancer stem cells in said subject that express IL-13R α 2 is measured. In another specific embodiment, the amount of cancer stem cells in said subject that express CD133 is measured. In another specific embodiment, the amount of cancer stem cells in said subject that express IL-13R α 2 and CD133 is measured.

[0009] In another aspect, provided herein are methods for monitoring the efficacy of an IL-13R α 2 peptide-based cancer treatment (i.e., a treatment or therapy that comprises administration of a peptide derived from IL-13R α 2) for a patient with cancer, comprising monitoring the amount of cancer stem cells in said subject prior to, during, and/or following the cancer treatment of a patient. In a specific embodiment, the amount of cancer stem cells in said subject that express IL-13R α 2 is measured. In another specific embodiment, the amount of cancer stem cells in said subject that express CD 133 is measured. In another specific embodiment, the amount of cancer stem cells in said subject that express IL-13R α 2 and CD133 is measured.

[0010] In another aspect, provided herein are methods for treating cancer comprising administering a T cell epitope

of IL-13R α 2 that targets cancer stem cells, wherein said epitope is sufficient to induce an immune response in a patient with cancer.

[0011] In another aspect, provided herein are methods of treating cancer in a subject, comprising administering to said subject a therapeutically effective amount of a compound that targets the IL-13R α 2 protein, wherein said compound is capable of killing and/or preventing the differentiation of cancer stem cells that express the IL-13R α 2 protein. In a specific embodiment, the compound is an antibody that specifically binds IL-13R α 2.

[0012] In another aspect, provided herein are methods of improving the targeting of cancer stem cells with a cancer vaccine comprising determining the binding motif of a Class I or Class II epitope from IL-13R α 2, and making substitutions in the amino acid sequence such that the modified peptides are able to induce an immune response that is at least as effective at killing cancer stem cells as the wild type peptide.

3.1 DEFINITIONS

[0013] As used herein, the terms "about" or "approximately" when used in conjunction with a number refer to any number within 1, 5 or 10% of the referenced number.

[0014] As used herein, the term "agent" refers to any molecule, compound, and/or substance that can be used in or in combination with a method treatment described herein. The term agent includes, without limitation, proteins, immunoglobulins (e.g., multi-specific Igs, single chain Igs, Ig fragments, polyclonal antibodies and their fragments, monoclonal antibodies and their fragments), peptides (e.g., peptide receptors, selectins), binding proteins, biologics, chemospecific agents, chemotoxic agents, anti-angiogenic agents, and small molecule drugs.

[0015] As used herein, the term "peptide" refers to a polymer of amino acids linked by amide bonds as is known to those of skill in the art. A peptide can be a polymer of 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more amino acids linked by covalent amide bonds. In some embodiments, the peptide is a polymer of 6 to 8, 8 to 10, 10 to 15, 10 to 20, 10 to 25, 10 to 30, 10 to 40, 10 to 50, or 25 to 25 amino acids linked by covalent amide bonds. In certain embodiments, the peptide is a polymer of 50 to 65, 50 to 75, 50 to 85, 50 to 95, 50 to 100, 75 to 100 amino acids linked by covalent amide bonds. As used herein, the term can refer to a single peptide chain linked by covalent amide bonds. The term can also refer to multiple peptide chains associated by non-covalent interactions such as ionic contacts, hydrogen bonds, Van der Waals contacts and hydrophobic contacts. Those of skill in the art will recognize that the term includes peptides that have been modified, for example by posttranslational processing such as signal peptide cleavage, disulfide bond formation, glycosylation (e.g., N-linked glycosylation), protease cleavage and lipid modification (e.g. S-palmitoylation).

[0016] As used herein, the terms "purified" and "isolated" when used in the context of a peptide that is obtained from a natural source, e.g., cells, refers to a peptide which is substantially free of contaminating materials from the natural source, e.g., soil particles, minerals, chemicals from the environment, and/or cellular materials from the natural source, such as but not limited to cell debris, cell wall materials, membranes, organelles, the bulk of the nucleic acids, carbohydrates, proteins, and/or lipids present in cells. Thus, a peptide that is isolated includes preparations of a polypeptide having less than about 30%, 20%, 10%, 5%, 2%, or 1% (by dry weight) of cellular materials and/or contaminating materials. As used herein, the terms "purified" and "isolated" when used in the context of a peptide that is chemically synthesized refers to a peptide which is substantially free of chemical precursors or other chemicals which are involved in the syntheses of the polypeptide.

[0017] As used herein, the term "nucleic acid" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid can be single-stranded or double-stranded.

[0018] As used herein, the term "therapeutically effective regimen" refers to a regimen for dosing, timing, frequency, and duration of the administration of one or more therapies for the treatment and/or management of cancer or a symptom thereof.

[0019] As used herein, the terms "subject" or "patient" are used interchangeably to refer to an animal (e.g., birds, reptiles, and mammals). In a specific embodiment, a subject is a bird. In another embodiment, a subject is a mammal including a non-primate (e.g., a camel, donkey, zebra, cow, pig, horse, goat, sheep, cat, dog, rat, and mouse) and a primate (e.g., a monkey, chimpanzee, and a human). In certain embodiments, a subject is a non-human animal. In some embodiments, a subject is a farm animal or pet. In another embodiment, a subject is a human. In another embodiment, a subject is a human infant. In another embodiment, a subject is a human toddler. In another embodiment, a subject is a human child. In another embodiment, a subject is a human adult. In another embodiment, a subject is an elderly human.

[0020] As used herein, the term "brain cancer" refers to a tumor located inside the cranium or in the central spinal canal. Brain cancer refers to both primary tumors (i.e., tumors that originate in the intracranial sphere or the central spinal canal) and secondary tumors (i.e., tumors that invaded the intracranial sphere or the central spinal canal after originating from tumors primarily located in other organs).

[0021] As used herein, the terms "therapies" and "therapy" can refer to any protocol(s), method(s), composition(s),

formulation(s), and/or agent(s) that can be used in the prevention or treatment of brain cancer or a disease or symptom associated therewith. In certain embodiments, the terms "therapies" and "therapy" refer to biological therapy, supportive therapy, and/or other therapies useful in treatment or prevention of cancer or a disease or symptom associated therewith known to one of skill in the art.

[0022] As used herein, the term "therapeutically effective amount" refers to the amount of a therapy that is sufficient to result in the prevention of the development, recurrence, or onset of cancer and one or more symptoms thereof, to enhance or improve the prophylactic effect(s) of another therapy, reduce the severity, the duration of cancer, ameliorate one or more symptoms of cancer, prevent the advancement of cancer, cause regression of cancer, and/or enhance or improve the therapeutic effect(s) of another therapy. In one embodiment, the amount of a therapeutically effective amount is effective to achieve one, two, three, or more results following the administration of one, two, three or more therapies: (1) a stabilization, reduction or elimination of the cancer stem cell population; (2) a stabilization, reduction or elimination in the cancer cell population; (3) a stabilization or reduction in the growth of a tumor or neoplasm; (4) an impairment in the formation of a tumor; (5) eradication, removal, or control of primary, regional and/or metastatic cancer; (6) a reduction in mortality; (7) an increase in disease-free, relapse-free, progression-free, and/or overall survival, duration, or rate; (8) an increase in the response rate, the durability of response, or number of patients who respond or are in remission; (9) a decrease in hospitalization rate, (10) a decrease in hospitalization lengths, (11) the size of the tumor is maintained and does not increase or increases by less than 10%, preferably less than 5%, preferably less than 4%, preferably less than 2%, (12) an increase in the number of patients in remission, (13) an increase in the length or duration of remission, (14) a decrease in the recurrence rate of cancer, (15) an increase in the time to recurrence of cancer, and (16) an amelioration of cancer-related symptoms and/or quality of life.

[0023] As used herein, the term "in combination" in the context of the administration of a therapy to a subject refers to the use of more than one therapy (e.g., prophylactic and/or therapeutic). The use of the term "in combination" does not restrict the order in which the therapies (e.g., a first and second therapy) are administered to a subject. A therapy can be administered prior to (e.g., 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy to a subject which had, has, or is susceptible to brain cancer. The therapies are administered to a subject in a sequence and within a time interval such that the therapies can act together. In a particular embodiment, the therapies are administered to a subject in a sequence and within a time interval such that they provide an increased benefit than if they were administered otherwise. Any additional therapy can be administered in any order with the other additional therapy.

[0024] As used herein, the terms "manage," "managing," and "management" in the context of the administration of a therapy to a subject refer to the beneficial effects that a subject derives from a therapy (e.g., a prophylactic or therapeutic vaccine) or a combination of therapies, while not resulting in a cure of cancer. In certain embodiments, a subject is administered one or more therapies (e.g., one or more prophylactic or therapeutic vaccines) to "manage" cancer so as to prevent the progression or worsening of the condition.

[0025] As used herein, the terms "prevent," "preventing" and "prevention" in the context of the administration of a therapy to a subject refer to the prevention or inhibition of the recurrence, onset, and/or development of brain cancer or a symptom thereof in a subject resulting from the administration of a therapy (e.g., a prophylactic or therapeutic agent), or a combination of therapies (e.g., a combination of prophylactic or therapeutic agents).

[0026] As used herein, the term "concurrently" means sufficiently close in time to produce a combined effect (that is, concurrently may be simultaneously, or it may be two or more events occurring within a time period before or after each other). When administered with other agents, the EphA2 and/or IL-13R α 2 peptides provided herein may be administered concurrently with the other active agent. In some embodiments the EphA2 and/or IL-13R α 2 peptides provided herein and one or more other agents (e.g., a helper T cell epitope, an adjuvant, and/or an immune response modifier) are administered to a subject concurrently, wherein administration of the EphA2 and/or IL-13R α 2 peptide and one or more other agents are in the same composition. In other embodiments an EphA2 and/or IL-13R α 2 peptide and one or more other agents (e.g., a helper T cell epitope, an adjuvant, and/or an immune response modifier) are administered to a subject concurrently, wherein administration of the EphA2 and/or IL-13R α 2 peptide and one or more other agents are not in the same composition. In certain embodiments, an EphA2 peptide and/or IL-13R α 2 provided herein and one or more other agents (e.g., a helper T cell epitope, an adjuvant, and/or an immune response modifier) are administered to a subject concurrently, wherein the concurrent administration is separated by at least 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 10 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, or 2 weeks.

[0027] As used herein, the term "EphA2 peptide" refers to a peptide derived from the EphA2 protein. In a specific embodiment the EphA2 protein from which an EphA2 peptide is derived is the human EphA2 protein. In another specific embodiment, an EphA2 peptide comprises or consists of the following amino acid sequence: TLADFDPRV (SEQ ID NO: 1). In some embodiments, an EphA2 peptide comprises one, two, three, or more amino acid mutations (e.g.,

additions, substitutions, or deletions) relative to the EphA2 peptide as it exists in the native (e.g., wild-type) form of the EphA2 protein.

[0028] As used herein, the term "IL-13R α 2 peptide" refers to a peptide derived from the IL-13R α 2 protein. In a specific embodiment the IL-13R α 2 protein from which an IL-13R α 2 peptide is derived is the human IL-13R α 2 protein. In another specific embodiment, an IL-13R α 2 peptide comprises or consists of the following amino acid sequence: WLPFGFILV (SEQ ID NO:2). In another specific embodiment, an IL-13R α 2 peptide comprises or consists of the following amino acid sequence: WLPFGFILV (SEQ ID NO:3). In another specific embodiment, an IL-13R α 2 peptide comprises or consists of the following amino acid sequence: ALPFGFILV (SEQ ID NO:4). In another specific embodiment, an IL-13R α 2 peptide comprises or consists of the following amino acid sequence: ELPFGFILV (SEQ ID NO:5). In some embodiments, an IL-13R α 2 peptide comprises one, two, three, or more amino acid mutations (e.g., additions, substitutions, or deletions) relative to the IL-13R α 2 peptide as it exists in the native (e.g., wild-type) form of the IL-13R α 2 protein.

[0029] As used herein and unless otherwise specified, the term "antibody" refers to a molecule with an antigen binding site that immunospecifically binds an antigen. Antibodies include, but are not limited to, monoclonal antibodies, polyclonal antibodies, recombinantly produced antibodies, multispecific antibodies (including bi-specific antibodies), human antibodies, humanized antibodies, chimeric antibodies, synthetic antibodies, tetrameric antibodies comprising two heavy chain and two light chain molecule, an antibody light chain monomer, an antibody heavy chain monomer, an antibody light chain dimer, an antibody heavy chain dimer, an antibody light chain- antibody heavy chain pair, intrabodies, heteroconjugate antibodies, single domain antibodies, monovalent antibodies, single-chain Fvs (scFv) (e.g., including monospecific, bispecific, etc.), camelized antibodies, Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), anti-idiotypic (anti-Id) antibodies (including, e.g., anti-anti-Id antibodies), and epitope-binding fragments of any of the above. Antibodies can be of any type (e.g., IgG, IgE, IgM, IgD, IgA or IgY), any class, (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 or IgA2), or any subclass (e.g., IgG2a or IgG2b) of immunoglobulin molecule. In certain embodiments, antibodies described herein are IgG antibodies, or a class (e.g., human IgG1 or IgG4) or subclass thereof.

4. BRIEF DESCRIPTION OF THE DRAWINGS

[0030]

Fig. 1 demonstrates that the bulk of the cells of the A-172 cancer cell line express EphA2 and IL-13R α 2 at high levels, but only a fraction of these cells express CD133.

Fig. 2 depicts joint staining of CD133 and EphA2 cells of the A-172 cancer cell line, and demonstrates that CD133+ cells of the cell line also express EphA2.

Fig. 3 depicts joint staining of CD133 and IL-13R α 2 cells of the A-172 cancer cell line, and demonstrates that CD133+ cells of the cell line also express IL-13R α 2.

Fig. 4 shows that CD133+ cells of the A-172 cancer cell line also express EphA2.

Fig. 5 shows that CD133+ cells of the A-172 cancer cell line also express IL-13R α 2.

Fig. 6 demonstrates that the bulk of the cells of the A-172 cancer cell line express EphA2 and IL-13R α 2 at high levels, but only a fraction of these cells express CD133.

Fig. 7 demonstrates that only a fraction of the cells of the A-172 cancer cell line express CD133.

Fig. 8 demonstrates that CD133+ cells of the A-172 cancer cell line also express EphA2 and IL-13R α 2.

5. DETAILED DESCRIPTION

[0031] In one aspect, provided herein are methods of treating cancer in a subject, comprising administering to said subject a therapeutically effective amount of a peptide derived from the EphA2 protein and monitoring the amount of cancer stem cells in said subject. In a specific embodiment, the amount of cancer stem cells in said subject that express EphA2 is measured. In another specific embodiment, the amount of cancer stem cells in said subject that express CD133 is measured. In another specific embodiment, the amount of cancer stem cells in said subject that express EphA2 and CD133 is measured.

[0032] In another aspect, provided herein are methods for monitoring the efficacy of an EphA2 peptide-based cancer treatment (i.e., a treatment or therapy that comprises administration of a peptide derived from EphA2) for a patient with cancer, comprising monitoring the amount of cancer stem cells in said subject prior to, during, and/or following the cancer treatment of a patient. In a specific embodiment, the amount of cancer stem cells in said subject that express EphA2 is measured. In another specific embodiment, the amount of cancer stem cells in said subject that express CD133 is measured. In another specific embodiment, the amount of cancer stem cells in said subject that express EphA2 and CD133 is measured.

[0033] In another aspect, provided herein are methods for treating cancer comprising administering a T cell epitope of EphA2 that targets cancer stem cells, wherein said epitope is sufficient to induce an immune response in a patient

with cancer.

[0034] In another aspect, provided herein are methods of treating cancer in a subject, comprising administering to said subject a therapeutically effective amount of a compound that targets the EphA2 protein, wherein said compound is capable of killing and/or preventing the differentiation of cancer stem cells that express the EphA2 protein. In a specific embodiment, the compound is an antibody that specifically binds EphA2.

[0035] In another aspect, provided herein are methods of improving the targeting of cancer stem cells with a cancer vaccine comprising determining the binding motif of a Class I or Class II epitope from EphA2, and making substitutions in the amino acid sequence such that the modified peptides are able to induce an immune response that is at least as effective at killing cancer stem cells as the wild type peptide.

[0036] In another aspect, provided herein are methods of treating cancer in a subject, comprising administering to said subject a therapeutically effective amount of a peptide derived from the IL-13R α 2 protein and monitoring the amount of cancer stem cells in said subject. In a specific embodiment, the amount of cancer stem cells in said subject that express IL-13R α 2 is measured. In another specific embodiment, the amount of cancer stem cells in said subject that express CD 133 is measured. In another specific embodiment, the amount of cancer stem cells in said subject that express IL-13R α 2 and CD133 is measured.

[0037] In another aspect, provided herein are methods for monitoring the efficacy of an IL-13R α 2 peptide-based cancer treatment (i.e., a treatment or therapy that comprises administration of a peptide derived from IL-13R α 2) for a patient with cancer, comprising monitoring the amount of cancer stem cells in said subject prior to, during, and/or following the cancer treatment of a patient. In a specific embodiment, the amount of cancer stem cells in said subject that express IL-13R α 2 is measured. In another specific embodiment, the amount of cancer stem cells in said subject that express CD133 is measured. In another specific embodiment, the amount of cancer stem cells in said subject that express IL-13R α 2 and CD133 is measured.

[0038] In another aspect, provided herein are methods for treating cancer comprising administering a T cell epitope of IL-13R α 2 that targets cancer stem cells, wherein said epitope is sufficient to induce an immune response in a patient with cancer.

[0039] In another aspect, provided herein are methods of treating cancer in a subject, comprising administering to said subject a therapeutically effective amount of a compound that targets the IL-13R α 2 protein, wherein said compound is capable of killing and/or preventing the differentiation of cancer stem cells that express the IL-13R α 2 protein. In a specific embodiment, the compound is an antibody that specifically binds IL-13R α 2.

[0040] In another aspect, provided herein are methods of improving the targeting of cancer stem cells with a cancer vaccine comprising determining the binding motif of a Class I or Class II epitope from IL-13R α 2, and making substitutions in the amino acid sequence such that the modified peptides are able to induce an immune response that is at least as effective at killing cancer stem cells as the wild type peptide.

5.1 METHODS OF MONITORING CANCER STEM CELLS

[0041] As part of the prophylactically effective and/or therapeutically effective regimens described herein, cancer stem cells can be monitored to assess the efficacy of an EphA2 peptide based cancer therapy or an IL-13R α 2 peptide based cancer therapy as well as to determine prognosis of a subject with cancer or the efficacy of a therapeutically or prophylactically effective regimen. In certain embodiments of the prophylactically effective and/or therapeutically effective therapies or regimens described herein, the therapies or regimens result in a stabilization or reduction of cancer stem cells in the patient. In one embodiment, the subject undergoing the regimen is monitored to assess whether the regimen has resulted in a stabilization or reduction in the cancer stem cells in the subject. In specific embodiments, the methods of monitoring measure an EphA2-, IL-13R α 2-, and/or CD133-expressing cancer stem cells in the subjects to whom an EphA2 peptide based cancer therapy or an IL-13R α 2 peptide based cancer therapy is administered.

[0042] Without being limited by any particular theory or mechanism of action, cancer stem cells, e.g., EphA2-expressing cancer stem cells and/or IL-13R α 2-expressing cancer stem cells, comprise a unique subpopulation (e.g., 0.1-10% or so) of a tumor that, in contrast to the remaining 90% or so of the tumor (i.e., the tumor bulk), are relatively more tumorigenic and relatively more slow-growing or quiescent. Given that conventional therapies and regimens have, in large part, been designed to attack rapidly proliferating cells (i.e., those cancer cells that comprise the tumor bulk), slower growing cancer stem cells may be relatively more resistant than faster growing tumor bulk to conventional therapies and regimens. This would explain another reason for the failure of standard oncology treatment regimens to ensure long-term benefit in most patients with advanced stage cancers. In a specific embodiment, an EphA2-expressing cancer stem cell or IL-13R α 2-expressing cancer stem cell is the founder cell of a tumor (i.e., it is the progenitor of cancer cells). In some embodiments, an EphA2-expressing cancer stem cell or IL-13R α 2-expressing cancer stem cell has one, two, three, or more or all of the following characteristics or properties: (i) can harbor the ability to initiate a tumor and/or to perpetuate tumor growth, (ii) can be generally relatively less mutated than the bulk of a tumor (e.g. due to slower growth and thus fewer DNA replication-dependent errors, improved DNA repair, and/or epigenetic/non-mutagenic changes contributing

to their malignancy), (iii) can have many features of a normal stem cell(s) (e.g., similar cell surface antigen and/or intracellular expression profile, self-renewal programs, multi-drug resistance, an immature phenotype, etc., characteristic of normal stem cells) and may be derived from a normal stem cell(s), (iv) can be potentially responsive to its microenvironment (e.g., the cancer stem cells may be capable of being induced to differentiate and/or divide asymmetrically), (v) can be the source of metastases, (vi) can be slow-growing or quiescent, (vii) can be symmetrically-dividing, (viii) can be tumorigenic (e.g. as determined by NOD/SCID implantation experiments), (ix) can be relatively resistant to traditional therapies (i.e. chemoresistant), and (x) can comprise a subpopulation of a tumor (e.g. relative to the tumor bulk).

[0043] In certain embodiments, the amount of cancer stem cells in a sample from a subject is determined/assessed using a technique described herein or well-known to one of skill in the art. Such samples include, but are not limited to, biological samples and samples derived from a biological sample. In certain embodiments, in addition to the biological sample itself or in addition to material derived from the biological sample such as cells, the sample used in the methods of this invention comprises added water, salts, glycerin, glucose, an antimicrobial agent, paraffin, a chemical stabilizing agent, heparin, an anticoagulant, or a buffering agent. In certain embodiments, the biological sample is blood, serum, urine, bone marrow or interstitial fluid. In another embodiment, the sample is a tissue sample. In a particular embodiment, the tissue sample is breast, brain, skin, colon, lung, liver, ovarian, pancreatic, prostate, renal, bone or skin tissue. In a specific embodiment, the tissue sample is a biopsy of normal or tumor tissue. The amount of biological sample taken from the subject will vary according to the type of biological sample and the method of detection to be employed. In a particular embodiment, the biological sample is blood, serum, urine, or bone marrow and the amount of blood, serum, urine, or bone marrow taken from the subject is 0.1 ml, 0.5 ml, 1 ml, 5 ml, 8 ml, 10 ml or more. In another embodiment, the biological sample is a tissue and the amount of tissue taken from the subject is less than 10 milligrams, less than 25 milligrams, less than 50 milligrams, less than 1 gram, less than 5 grams, less than 10 grams, less than 50 grams, or less than 100 grams.

[0044] In accordance with the methods described herein, a sample derived from a biological sample is one in which the biological sample has been subjected to one or more pretreatment steps prior to the detection and/or measurement of the cancer stem cell population in the sample. In certain embodiments, a biological fluid is pretreated by centrifugation, filtration, precipitation, dialysis, or chromatography, or by a combination of such pretreatment steps. In other embodiments, a tissue sample is pretreated by freezing, chemical fixation, paraffin embedding, dehydration, permeabilization, or homogenization followed by centrifugation, filtration, precipitation, dialysis, or chromatography, or by a combination of such pretreatment steps. In certain embodiments, the sample is pretreated by removing cells other than stem cells or cancer stem cells from the sample, or removing debris from the sample prior to the determination of the amount of cancer stem cells in the sample according to the methods of the invention.

[0045] The samples for use in the methods of described herein may be taken from any animal subject, preferably mammal, most preferably a human. The subject from which a sample is obtained and utilized in accordance with the methods of this invention includes, without limitation, an asymptomatic subject, a subject manifesting or exhibiting 1, 2, 3, 4 or more symptoms of cancer, a subject clinically diagnosed as having cancer, a subject predisposed to cancer, a subject suspected of having cancer, a subject undergoing therapy for cancer, a subject that has been medically determined to be free of cancer (e.g., following therapy for the cancer), a subject that is managing cancer, or a subject that has not been diagnosed with cancer. In certain embodiments, the term "has no detectable cancer," as used herein, refers to a subject or subjects in which there is no detectable cancer by conventional methods, e.g., MRI. In other embodiments, the term refers to a subject or subjects free from any disorder.

[0046] In certain embodiments, the amount of cancer stem cells in a subject or a sample from a subject is/are assessed prior to therapy or regimen (e.g. at baseline) or at least 1, 2, 4, 6, 7, 8, 10, 12, 14, 15, 16, 18, 20, 30, 60, 90 days, 6 months, 9 months, 12 months, or > 12 months after the subject begins receiving the therapy or regimen. In certain embodiments, the amount of cancer stem cells is assessed after a certain number of doses (e.g., after 2, 5, 10, 20, 30 or more doses of a therapy). In other embodiments, the amount of cancer stem cells is assessed after 1 week, 2 weeks, 1 month, 2 months, 1 year, 2 years, 3 years, 4 years or more after receiving one or more therapies.

[0047] In certain embodiments, a positive or negative control sample is a sample that is obtained or derived from a corresponding tissue or biological fluid or tumor as the sample to be analyzed in accordance with the methods of the invention. This sample may come from the same patient or different persons and at the same or different time points.

[0048] For clarity of disclosure, and not by way of limitation, the following pertains to analysis of a blood sample from a patient. However, as one skilled in the art will appreciate, the assays and techniques described herein can be applied to other types of patient samples, including a body fluid (e.g. blood, bone marrow, plasma, urine, bile, ascitic fluid), a tissue sample suspected of containing material derived from a cancer (e.g. a biopsy) or homogenate thereof. The amount of sample to be collected will vary with the particular type of sample and method of determining the amount of cancer stem cells used and will be an amount sufficient to detect the cancer stem cells in the sample.

[0049] A sample of blood may be obtained from a patient having different developmental or disease stages. Blood may be drawn from a subject from any part of the body (e.g., a finger, a hand, a wrist, an arm, a leg, a foot, an ankle, a stomach, and a neck) using techniques known to one of skill in the art, in particular methods of phlebotomy known in

the art. In a specific embodiment, venous blood is obtained from a subject and utilized in accordance with the methods of the invention. In another embodiment, arterial blood is obtained and utilized in accordance with the methods of the invention. The composition of venous blood varies according to the metabolic needs of the area of the body it is servicing. In contrast, the composition of arterial blood is consistent throughout the body. For routine blood tests, venous blood is generally used.

[0050] The amount of blood collected will vary depending upon the site of collection, the amount required for a method of the invention, and the comfort of the subject. In some embodiments, any amount of blood is collected that is sufficient to detect the amount of cancer stem cells. In a specific embodiment, 1cc or more of blood is collected from a subject.

[0051] The amount of cancer stem cells in a sample can be expressed as the percentage of, e.g., overall cells, overall cancer cells or overall stem cells in the sample, or quantitated relative to area (e.g. cells per high power field), or volume (e.g. cells per ml), or architecture (e.g. cells per bone spicule in a bone marrow specimen).

[0052] In some embodiments, the sample may be a blood sample, bone marrow sample, or a tissue/tumor biopsy sample, wherein the amount of cancer stem cells per unit of volume (e.g., 1 mL) or other measured unit (e.g., per unit field in the case of a histological analysis) is quantitated. In certain embodiments, the cancer stem cell population is determined as a portion (e.g., a percentage) of the cancerous cells present in the blood or bone marrow or tissue/tumor biopsy sample or as a subset of the cancerous cells present in the blood or bone marrow or tissue/tumor biopsy sample. The cancer stem cell population, in other embodiments, can be determined as a portion (e.g., percentage) of the total cells. In yet other embodiments, the cancer stem cell population is determined as a portion (e.g., a percentage) of the total stem cells present in the blood sample.

[0053] In other embodiments, the sample from the patient is a tissue sample (e.g., a biopsy from a subject with or suspected of having cancerous tissue), where the amount of cancer stem cells can be measured, for example, by immunohistochemistry or flow cytometry, or on the basis of the amount of cancer stem cells per unit area, volume, or weight of the tissue. In certain embodiments, the cancer stem cell population (the amount of cancer stem cells) is determined as a portion (e.g., a percentage) of the cancerous cells present in the tissue sample or as a subset of the cancerous cells present in the tissue sample. In yet other embodiments, the cancer stem cell population is determined as a portion (e.g., a percentage) of the overall cells or stem cell cells in the tissue sample.

[0054] The amount of cancer stem cells in a test sample can be compared with the amount of cancer stem cells in reference sample(s) to assess the efficacy of the regimen. In one embodiment, the reference sample is a sample obtained from the subject undergoing therapy at an earlier time point (e.g., prior to receiving the regimen as a baseline reference sample, or at an earlier time point while receiving the therapy). In this embodiment, the therapy desirably results in a decrease in the amount of cancer stem cells in the test sample as compared with the reference sample. In another embodiment, the reference sample is obtained from a healthy subject who has no detectable cancer, or from a patient that is in remission for the same type of cancer. In this embodiment, the therapy desirably results in the test sample having an equal amount of cancer stem cells, or less than the amount of cancer stem cells than are detected in the reference sample.

[0055] In other embodiments, the cancer stem cell population in a test sample can be compared with a predetermined reference range and/or a previously detected amount of cancer stem cells determined for the subject to gauge the subject's response to the regimens described herein. In a specific embodiment, a stabilization or reduction in the amount of cancer stem cells relative to a predetermined reference range and/or earlier (previously detected) cancer stem cell amount determined for the subject indicates an improvement in the subject's prognosis or a positive response to the regimen, whereas an increase relative to the predetermined reference range and/or earlier cancer stem cell amount indicates the same or worse prognosis, and/or a failure to respond to the regimen. The cancer stem cell amount can be used in conjunction with other measures to assess the prognosis of the subject and/or the efficacy of the regimen. In a specific embodiment, the predetermined reference range is based on the amount of cancer stem cells obtained from a patient or population(s) of patients suffering from the same type of cancer as the patient undergoing the therapy.

[0056] Generally, since stem cell antigens, e.g., EphA2, CD133, and IL-13R α 2, can be present on both cancer stem cells and normal stem cells, a sample from the cancer-afflicted patient will have a higher stem cell count than a sample from a healthy subject who has no detectable cancer, due to the presence of the cancer stem cells. The therapy will desirably result in a cancer stem cell count for the test sample (e.g., the sample from the patient undergoing therapy) that decreases and becomes increasingly closer to the stem cell count in a reference sample that is sample from a healthy subject who has no detectable cancer.

[0057] If the reduction in amount of cancer stem cells is determined to be inadequate upon comparing the amount of cancer stem cells in the sample from the subject undergoing the regimen with the reference sample, then the medical practitioner has a number of possible options to adjust the regimen. For instance, the medical practitioner can then increase either the dosage or intensity of the therapy administered, the frequency of the administration, the duration of administration, combine the therapy with another therapy(ies), change the management altogether including halting therapy, or any combination thereof.

[0058] In certain embodiments, the dosage, frequency and/or duration of administration of a therapy is modified as a

result of the change in the amount of cancer stem cells detected in or from the treated patient. For example, if a subject receiving therapy for leukemia has an cancer stem cell measurement of 2.5% of his tumor prior to therapy and 5% after 6 weeks of therapy, then the therapy or regimen may be altered or stopped because the increase in the percentage of cancer stem cells indicates that the therapy or regimen is not optimal. Alternatively, if another subject has an cancer stem cell measurement of 2.5% of his tumor prior to therapy and 1% after 6 weeks of therapy, then the therapy or regimen may be continued because the decrease in the percentage of cancer stem cells indicates that the therapy or regimen is effective.

[0059] The amount of cancer stem cells can be monitored/assessed using standard techniques known to one of skill in the art. Cancer stem cells can be monitored by, e.g., obtaining a sample, such as a tissue/tumor sample, blood sample or a bone marrow sample, from a subject and detecting cancer stem cells in the sample. The amount of cancer stem cells in a sample (which may be expressed as percentages of, e.g., overall cells or overall cancer cells) can be assessed by detecting the expression cancer stem cell antigens (e.g., EphA2) on cancer stem cells. Techniques known to those skilled in the art can be used for measuring these activities. Antigen expression can be assayed, for example, by immunoassays including, but not limited to, western blots, immunohistochemistry, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complementfixation assays, immunoradiometric assays, fluorescent immunoassays, immunofluorescence, protein A immunoassays, flow cytometry, and FACS analysis. In such circumstances, the amount of cancer stem cells in a test sample from a subject may be determined by comparing the results to the amount of cancer stem cells in a reference sample (e.g., a sample from a subject who has no detectable cancer) or to a predetermined reference range, or to the patient him/herself at an earlier time point (e.g. prior to, or during therapy).

[0060] In a specific embodiment, the cancer stem cell population in a sample from a patient is determined by flow cytometry. This method exploits the differential expression of certain surface markers on cells. Labeled antibodies (e.g., fluorescent antibodies) specific to cancer stem cells antigens (e.g., EphA2) can be used to react with the cells in the sample, and the cells are subsequently sorted by FACS methods. In some embodiments, a combination of cell surface markers are utilized in order to determine the amount of cancer stem cells in the sample. For example, both positive and negative cell sorting may be used to assess the amount of cancer stem cells in the sample. In a specific embodiment the cancer stem cell population in a sample, e.g., a tissue sample, such as a solid tumor biopsy, is determined using immunohistochemistry techniques. This method exploits the differential expression of certain surface markers on cells. Labeled antibodies (e.g., fluorescent antibodies) specific to cancer stem cells antigens (e.g., EphA2) can be used to react with the cells in the sample, and the tissue is subsequently stained. In some embodiments, a combination of certain cell surface markers are utilized in order to determine the amount of cancer stem cells in the sample.

[0061] In other embodiments, sphere formation can be used to determine the amount of cancer stem cells in a sample (See Singh et al., "Identification of a Cancer Stem Cell from Human Brain Tumors," Cancer Res 63: 5821-5828 (2003)).

[0062] In other embodiments, a sample (e.g., a tumor or normal tissue sample, blood sample or bone marrow sample) obtained from the patient is analyzed in *in vivo* systems to determine the cancer stem cell population or amount of cancer stem cells. In certain embodiments, for example, *in vivo* engraftment is used to quantitate the amount of cancer stem cells in a sample. *In vivo* engraftment involves implantation of a human specimen with the readout being the formation of tumors in an animal such as in immunocompromised or immunodeficient mice (such as NOD/SCID mice). Typically, the patient sample is cultured or manipulated *in vitro* and then injected into the mice. In these assays, mice can be injected with a decreasing amount of cells from patient samples, and the frequency of tumor formation can be plotted vs. the amount of cells injected to determine the amount of cancer stem cells in the sample. Alternatively, the rate of growth of the resulting tumor can be measured, with larger or more rapidly advancing tumors indicating a higher cancer stem cells amount in the patient sample. In this way, an *in vivo* engraftment model/assay could be used to measure cancer stem cells amount pre- and posttherapy to assess the change in cancer stem cell amount arising from a given therapy or regimen.

[0063] In certain *in vivo* techniques, an imaging agent or diagnostic agent is used which binds to biological molecules on cancer cells or cancer stem cells, e.g., binds to EphA2 on cancer stem cells. For instance, a fluorescent tag, radionuclide, heavy metal, or photon-emitter is attached to an antibody (including an antibody fragment) that binds to EphA2. The medical practitioner can infuse the labeled antibody into the patient either prior to, during, or following treatment, and then the practitioner can place the patient into a total body scanner/developer which can detect the attached label (e.g., fluorescent tag, radionuclide, heavy metal, photon-emitter). The scanner/developer (e.g., CT, MRI, or other scanner, e.g. detector of fluorescent label, that can detect the label) records the presence, amount/quantity, and bodily location of the bound antibody. In this manner, the mapping and quantitation of tag (e.g. fluorescence, radioactivity, etc.) in patterns (*i.e.*, different from patterns of normal stem cells within a tissue) within a tissue or tissues indicates the treatment efficacy within the patient's body when compared to a reference control such as the same patient at an earlier time point or a patient or healthy individual who has no detectable cancer. For example, a large signal (relative to a reference range or a prior treatment date, or prior to treatment) at a particular location indicates the presence of cancer stem cells. If this

signal is increased relative to a prior date it suggests a worsening of the disease and failure of therapy or regimen. Alternatively, a signal decrease indicates that the therapy or regimen has been effective.

[0064] In a specific embodiment, the amount of cancer stem cells is detected in vivo in a subject according to a method comprising the steps of: (a) administering to the subject an effective amount of a labeled binding agent that specifically binds to an antigen of cancer stem cells (e.g., EphA2 or CD133), and (b) detecting the labeled agent in the subject following a time interval sufficient to allow the labeled agent to concentrate at sites in the subject where the cancer stem cell surface marker is expressed. In accordance with this embodiment, the binding agent is administered to the subject according to any suitable method in the art, for example, parenterally (such as intravenously), or intraperitoneally. In accordance with this embodiment, the effective amount of the agent is the amount which permits the detection of the agent in the subject. This amount will vary according to the particular subject, the label used, and the detection method employed. For example, it is understood in the art that the size of the subject and the imaging system used will determine the amount of labeled agent needed to detect the agent in a subject using an imaging means. In the case of a radiolabeled agent for a human subject, the amount of labeled agent administered is measured in terms of radioactivity, for example from about 5 to 20 millicuries of ⁹⁹Tc. The time interval following the administration of the labeled agent which is sufficient to allow the labeled agent to concentrate at sites in the subject where the cancer stem cell surface marker is expressed will vary depending on several factors, for example, the type of label used, the mode of administration, and the part of the subject's body that is imaged. In a particular embodiment, the time interval that is sufficient is 6 to 48 hours, 6 to 24 hours, or 6 to 12 hours. In another embodiment the time interval is 5 to 20 days or 5 to 10 days. The presence of the labeled cancer stem cell surface marker-binding agent can be detected in the subject using imaging means known in the art. In general, the imaging means employed depend upon the type of label used. Skilled artisans will be able to determine the appropriate means for detecting a particular label. Methods and devices that may be used include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), an imager which can detect and localize fluorescent label, and sonography. In a specific embodiment, the cancer binding agent is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the binding agent is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the binding agent is labeled with a positron emitting metal and is detected in the patient using positron emission tomography. In yet another embodiment, the binding agent is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

[0065] Any *in vitro* or *in vivo* (ex vivo) assays known to those skilled in the art that can detect and/or quantify cancer stem cells can be used to monitor cancer stem cells in or from a subject in order to evaluate the prophylactic and/or therapeutic utility of a cancer therapy or regimen disclosed herein for cancer or one or more symptoms thereof; or these assays can be used to assess the prognosis of a patient. The results of these assays then may be used to possibly maintain or alter the cancer therapy or regimen.

[0066] The amount of cancer stem cells in a specimen can be compared to a predetermined reference range and/or an earlier amount of cancer stem cells previously determined for the subject (either prior to, or during therapy) in order to gauge the subject's response to the treatment regimens described herein. In a specific embodiment, a stabilization or reduction in the amount of cancer stem cells relative to a predetermined reference range and/or earlier cancer stem cell amount previously determined for the subject (either prior to, or during therapy) indicates that the therapy or regimen was effective and thus possibly an improvement in the subject's prognosis, whereas an increase relative to the predetermined reference range and/or cancer stem cell amount detected at an earlier time point indicates that the therapy or regimen was ineffective and thus possibly the same or a worsening in the subject's prognosis. The cancer stem cell amount can be used with other standard measures of cancer to assess the prognosis of the subject and/or efficacy of the therapy or regimen: such as response rate, durability of response, relapse-free survival, disease-free survival, progression-free survival, and overall survival. In certain embodiments, the dosage, frequency and/or duration of administration of a therapy is modified as a result of the determination of the amount or change in the amount of cancer stem cells at various time points which may include prior to, during, and/or following therapy.

[0067] Also provided herein are methods for determining that a cancer therapy or regimen is effective at targeting and/or impairing cancer stem cells by virtue of monitoring cancer stem cells over time and detecting a stabilization or decrease in the amount of cancer stem cells during and/or following the course of the cancer therapy or regimen.

[0068] In a certain embodiment, a therapy or regimen may be described or marketed as an anti-cancer stem cell therapy or regimen based on the determination that a therapy or regimen is effective at targeting and/or impairing cancer stem cells by virtue of having monitored or detected a stabilization or decrease in the amount of cancer stem cells during therapy.

[0069] Also provided herein are methods to treat cancer involving i) determining that an EphA2-based cancer therapy is effective by virtue of its ability to decrease cancer stem cells as determined by the monitoring of cancer stem cells, and ii) administering the therapy to a human(s) with cancer. Also provided herein are methods to methods to treat cancer involving i) administering to a human with cancer an EphA2-based cancer therapy, ii) determining the amount of cancer

stem cells prior to, during, and/or following therapy through the monitoring of cancer stem cells, and iii) continuing, altering, or halting therapy based on such monitoring. Also provided herein are methods for assaying/screening of an EphA2-based therapy(s) for anti-cancer stem cell activity involving i) administration of the therapy to a human with cancer, ii) monitoring cancer stem cells in or from the human prior to, during, and/or following therapy, and iii) determining whether the therapy resulted in a decrease in the amount of cancer stem cells.

[0070] Also provided herein are methods to treat cancer involving i) determining that an IL-13R α 2-based cancer therapy is effective by virtue of its ability to decrease cancer stem cells as determined by the monitoring of cancer stem cells, and ii) administering the therapy to a human(s) with cancer. Also provided herein are methods to methods to treat cancer involving i) administering to a human with cancer an IL-13R α 2-based cancer therapy, ii) determining the amount of cancer stem cells prior to, during, and/or following therapy through the monitoring of cancer stem cells, and iii) continuing, altering, or halting therapy based on such monitoring. Also provided herein are methods for assaying/screening of an IL-13R α 2-based therapy(s) for anti-cancer stem cell activity involving i) administration of the therapy to a human with cancer, ii) monitoring cancer stem cells in or from the human prior to, during, and/or following therapy, and iii) determining whether the therapy resulted in a decrease in the amount of cancer stem cells.

5.2 TYPES OF CANCER

[0071] With any type of cancer for which a patient can be treated, the cancer stem cells thereof can be monitored in accordance with the methods described herein. The medical practitioner can diagnose the patient using any of the conventional cancer screening methods including, but not limited to physical examination (e.g., prostate examination, rectal examination, breast examination, lymph nodes examination, abdominal examination, skin surveillance, testicular exam, general palpation), visual methods (e.g., colonoscopy, bronchoscopy, endoscopy), PAP smear analyses (cervical cancer), stool guaiac analyses, blood tests (e.g., complete blood count (CBC) test, prostate specific antigen (PSA) test, carcinoembryonic antigen (CEA) test, cancer antigen (CA)-125 test, alpha-fetoprotein (AFP), liver function tests), karyotyping analyses, bone marrow analyses (e.g., in cases of hematological malignancies), histology, cytology, flow cytometry, a sputum analysis and imaging methods (e.g., computed tomography (CT), magnetic resonance imaging (MRI), ultrasound, X-ray imaging, mammography, PET scans, bone scans).

[0072] Non-limiting examples of cancers include: leukemias, such as but not limited to, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemias, such as, myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia leukemias and myelodysplastic syndrome (MDS); chronic leukemias, such as but not limited to, chronic myelocytic (granulocytic) leukemia, chronic lymphocytic leukemia, hairy cell leukemia; polycythemia vera; lymphomas such as but not limited to Hodgkin's disease, non-Hodgkin's disease; multiple myelomas such as but not limited to smoldering multiple myeloma, nonsecretory myeloma, osteosclerotic myeloma, plasma cell leukemia, solitary plasmacytoma and extramedullary plasmacytoma; Waldenström's macroglobulinemia; monoclonal gammopathy of undetermined significance; benign monoclonal gammopathy; heavy chain disease; bone and connective tissue sarcomas such as but not limited to bone sarcoma, osteosarcoma, chondrosarcoma, Ewing's sarcoma, malignant giant cell tumor, fibrosarcoma of bone, chordoma, periosteal sarcoma, soft-tissue sarcomas, angiosarcoma (hemangiosarcoma), fibrosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, neurilemmoma, rhabdomyosarcoma, synovial sarcoma; brain tumors such as but not limited to, glioma, astrocytoma, brain stem glioma, ependymoma, oligodendroglioma, nonglial tumor, acoustic neurinoma, craniopharyngioma, medulloblastoma, meningioma, pineocytoma, pineoblastoma, primary brain lymphoma; breast cancer including but not limited to ductal carcinoma, adenocarcinoma, lobular (small cell) carcinoma, intraductal carcinoma, medullary breast cancer, mucinous breast cancer, tubular breast cancer, papillary breast cancer, Paget's disease, and inflammatory breast cancer; adrenal cancer such as but not limited to pheochromocytoma and adrenocortical carcinoma; thyroid cancer such as but not limited to papillary or follicular thyroid cancer, medullary thyroid cancer and anaplastic thyroid cancer; pancreatic cancer such as but not limited to, insulinoma, gastrinoma, glucagonoma, vipoma, somatostatin-secreting tumor, and carcinoid or islet cell tumor; pituitary cancers such as but limited to Cushing's disease, prolactin-secreting tumor, acromegaly, and diabetes insipidus; eye cancers such as but not limited to ocular melanoma such as iris melanoma, choroidal melanoma, and ciliary body melanoma, and retinoblastoma; vaginal cancers such as squamous cell carcinoma, adenocarcinoma, and melanoma; vulvar cancer such as squamous cell carcinoma, melanoma, adenocarcinoma, basal cell carcinoma, sarcoma, and Paget's disease; cervical cancers such as but not limited to, squamous cell carcinoma, and adenocarcinoma; uterine cancers such as but not limited to endometrial carcinoma and uterine sarcoma; ovarian cancers such as but not limited to, ovarian epithelial carcinoma, borderline tumor, germ cell tumor, and stromal tumor; esophageal cancers such as but not limited to, squamous cancer, adenocarcinoma, adenoid cystic carcinoma, mucoepidermoid carcinoma, adenosquamous carcinoma, sarcoma, melanoma, plasmacytoma, verrucous carcinoma, and oat cell (small cell) carcinoma; stomach cancers such as but not limited to, adenocarcinoma, fungating (polypoid), ulcerating, superficial spreading, diffusely spreading, malignant lymphoma, liposarcoma, fibrosarcoma, and carcinosarcoma; colon cancers; rectal cancers; liver cancers such as but not limited to hepatocellular carcinoma and hepatoblastoma; gallbladder cancers such as adeno-

carcinoma; cholangiocarcinomas such as but not limited to papillary, nodular, and diffuse; lung cancers such as non-small cell lung cancer, squamous cell carcinoma (epidermoid carcinoma), adenocarcinoma, large-cell carcinoma and small-cell lung cancer; testicular cancers such as but not limited to germinal tumor, seminoma, anaplastic, classic (typical), spermatocytic, nonseminoma, embryonal carcinoma, teratoma carcinoma, choriocarcinoma (yolk-sac tumor), prostate cancers such as but not limited to, prostatic intraepithelial neoplasia, adenocarcinoma, leiomyosarcoma, and rhabdomyosarcoma; penal cancers; oral cancers such as but not limited to squamous cell carcinoma; basal cancers; salivary gland cancers such as but not limited to adenocarcinoma, mucoepidermoid carcinoma, and adenoidcystic carcinoma; pharynx cancers such as but not limited to squamous cell cancer, and verrucous; skin cancers such as but not limited to, basal cell carcinoma, squamous cell carcinoma and melanoma, superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, acral lentiginous melanoma; kidney cancers such as but not limited to renal cell carcinoma, adenocarcinoma, hypernephroma, fibrosarcoma, transitional cell cancer (renal pelvis and/ or uterer); Wilms' tumor; bladder cancers such as but not limited to transitional cell carcinoma, squamous cell cancer, adenocarcinoma, carcinosarcoma. In addition, cancers include myxosarcoma, osteogenic sarcoma, endotheliosarcoma, lymphangioendotheliosarcoma, mesothelioma, synovioma, hemangioblastoma, epithelial carcinoma, cystadenocarcinoma, bronchogenic carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma and papillary adenocarcinomas (for a review of such disorders, see Fishman et al., 1985, Medicine, 2d Ed., J.B. Lippincott Co., Philadelphia and Murphy et al., 1997, Informed Decisions: The Complete Book of Cancer Diagnosis, Treatment, and Recovery, Viking Penguin, Penguin Books U.S.A., Inc., United States of America).

[0073] Other cancers or other abnormal proliferative diseases, include but are not limited to, the following: carcinoma, including that of the bladder, breast, colon, kidney, liver, lung, ovary, pancreas, stomach, cervix, thyroid and skin; including squamous cell carcinoma; hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T cell lymphoma, Burkitt's lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyosarcoma; other tumors, including melanoma, seminoma, teratocarcinoma, neuroblastoma and glioma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyosarcoma, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoactanthoma, seminoma, thyroid follicular cancer and teratocarcinoma. Cancers associated with aberrations in apoptosis are also included and are not be limited to, follicular lymphomas, carcinomas with p53 mutations, hormone dependent tumors of the breast, prostate and ovary, and precancerous lesions such as familial adenomatous polyposis, and myelodysplastic syndromes. In specific embodiments, malignancy or dysproliferative changes (such as metaplasias and dysplasias), or hyperproliferative disorders of the skin, lung, liver, bone, brain, stomach, colon, breast, prostate, bladder, kidney, pancreas, ovary, and/or uterus are encompassed in the invention.

[0074] Non-limiting examples of leukemias and other blood-borne cancers include acute lymphoblastic leukemia "ALL", acute lymphoblastic B-cell leukemia, acute lymphoblastic T-cell leukemia, acute myeloblastic leukemia "AML", acute promyelocytic leukemia "APL", acute monoblastic leukemia, acute erythroleukemic leukemia, acute megakaryoblastic leukemia, acute myelomonocytic leukemia, acute nonlymphocytic leukemia, acute undifferentiated leukemia, chronic myelocytic leukemia "CML", chronic lymphocytic leukemia "CLL", and hairy cell leukemia.

[0075] Non-limiting examples of lymphomas include Hodgkin's disease, non-Hodgkin's Lymphoma, Multiple myeloma, Waldenstrom's macroglobulinemia, Heavy chain disease, and Polycythemia vera.

[0076] Non-limiting examples of solid tumors encompassed in the invention include, but are not limited to fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon cancer, colorectal cancer, kidney cancer, pancreatic cancer, bone cancer, breast cancer, ovarian cancer, prostate cancer, esophageal cancer, stomach cancer, oral cancer, nasal cancer, throat cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, uterine cancer, testicular cancer, small cell lung carcinoma, bladder carcinoma, lung cancer, epithelial carcinoma, glioma, glioblastoma multiforme, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, skin cancer, melanoma, neuroblastoma, and retinoblastoma.

5.2.1 BRAIN CANCERS

[0077] In a specific embodiment, the methods described herein can be used in the prevention, treatment, and/or management of brain cancer. In certain embodiments, such methods comprise a step of monitoring the levels of cancer stem cells in a subject with brain cancer that has been treated in accordance with the methods described herein, e.g.,

the subject has been administered an EphA2-based cancer therapy, i.e., an EphA2 peptide or a compound (e.g., an antibody) that specifically targets EphA2 (e.g., targets EphA2 present on EphA2-expressing cancer stem cells).

[0078] In another specific embodiment, the methods described herein can be used in the prevention, treatment, and/or management of brain cancer. In certain embodiments, such methods comprise a step of monitoring the levels of cancer stem cells in a subject with brain cancer that has been treated in accordance with the methods described herein, e.g., the subject has been administered an IL-13R α 2-based cancer therapy, i.e., an IL-13R α 2 peptide or a compound (e.g., an antibody) that specifically targets IL-13R α 2 (e.g., targets IL-13R α 2 present on IL-13R α 2-expressing cancer stem cells).

[0079] Any type of brain cancer can be treated in accordance with the methods described herein. Exemplary brain cancers include, but are not limited to, gliomas (including astrocytoma (e.g., pilocytic astrocytoma, diffuse astrocytoma, and anaplastic astrocytoma), glioblastoma, oligodendroglioma, brain stem glioma, non-brain stem glioma, ependymoma, and mixed tumors comprising more than one glial cell types), acoustic schwannoma, cranialpharyngioma, meningioma, medulloblastoma, primary central nervous system lymphoma, and tumors of the pineal (e.g., pineal astrocytic tumors and pineal parenchymal tumors) and pituitary glands. Gliomas additionally include recurrent malignant gliomas, high-risk WHO Grade II Astrocytomas, Oligo Astrocytomas, recurrent WHO Grade II Gliomas, newly-diagnosed malignant or intrinsic brain stem gliomas, incompletely resected non-brainstem gliomas, and recurrent unresectable low-grade gliomas. Additional types of brain cancer that can be treated in accordance with the methods described herein include adult low-grade infiltrative supratentorial astrocytoma/oligodendroglioma, adult low-grade infiltrative supratentorial astrocytoma, adult low-grade infiltrative supratentorial oligodendroglioma, adult low-grade infiltrative supratentorial astrocytoma/oligodendroglioma (excluding pilocytic astrocytoma), adult low-grade infiltrative supratentorial astrocytoma (excluding pilocytic astrocytoma), adult low-grade infiltrative supratentorial oligodendroglioma (excluding pilocytic astrocytoma), adult intracranial ependymoma, adult intracranial ependymoma (excluding subependymoma and myxopapillary), adult intracranial anaplastic ependymoma, anaplastic glioma, anaplastic glioblastoma, pilocytic astrocytoma, subependymoma, myxopapillary, 1 to 3 limited metastatic lesions (intraparenchymal), greater than 3 metastatic lesions (intraparenchymal), leptomeningeal metastases (neoplastic meningitis), primary CNS lymphoma, metastatic spine tumors, or meningiomas.

[0080] In one embodiment, the brain cancer treated in accordance with the methods described herein is a glioma. In a specific embodiment, the brain cancer treated in accordance with the methods described herein is recurrent malignant glioma. In another specific embodiment, the brain cancer treated in accordance with the methods described herein is recurrent WHO Grade II Glioma. In another specific embodiment, the brain cancer treated in accordance with the methods described herein is newly-diagnosed malignant or intrinsic brain stem glioma. In another specific embodiment, the brain cancer treated in accordance with the methods described herein is incompletely resected non-brainstem glioma. In another specific embodiment, the brain cancer treated in accordance with the methods described herein is recurrent unresectable low-grade glioma.

[0081] In one embodiment, a patient treated in accordance with the methods described herein is an adult with recurrent malignant glioma, recurrent glioblastoma, anaplastic astrocytoma, anaplastic oligodendroglioma, or anaplastic mixed oligoastrocytoma. In another embodiment, the patient is an adult with newly diagnosed high-risk low grade glioma. In another embodiment, the patient is an adult with newly diagnosed high-risk low grade astrocytoma. In another embodiment, the patient is an adult with newly diagnosed high-risk low grade oligoastrocytoma. In another embodiment, the patient is an adult with recurrent high-risk low grade astrocytoma. In another embodiment, the patient is an adult with recurrent high-risk low grade oligoastrocytoma. In another embodiment, the patient is an adult with recurrent high-risk low grade oligodendroglioma. In another embodiment, the patient is a child with newly diagnosed malignant glioma. In another embodiment, the patient is a child with intrinsic brain stem glioma. In another embodiment, the patient is a child with incompletely resected non-brainstem high-grade glioma. In another embodiment, the patient is a child with recurrent unresectable low-grade glioma. In another embodiment, the patient is a child with newly diagnosed diffuse intrinsic pontine glioma. In another embodiment, the patient is a child with any high-grade glioma involving the brainstem and treated with RT or without chemotherapy during RT. In another embodiment, the patient is a child with newly diagnosed non-brainstem high-grade glioma treated with RT with chemotherapy. In another embodiment, the patient is a child with newly diagnosed non-brainstem high-grade glioma treated with RT without chemotherapy. In another embodiment, the patient is a child with recurrent non-brainstem high-grade glioma that has recurred after treatment.

[0082] In another embodiment, the brain cancer treated in accordance with the methods described herein is an astrocytoma. In a specific embodiment, the brain cancer treated in accordance with the methods described herein is high-risk WHO Grade II Astrocytoma. In another specific embodiment, the brain cancer treated in accordance with the methods described herein is Oligo Astrocytoma.

5.3 Peptides

5.3.1 Peptides derived from EphA2

[0083] EphA2 is a tyrosine kinase receptor that is involved in the formation of the notochord via interaction with ephrinA1. (see, e.g., Naruse-Nakajima et al., Mech. Dev., 102: 95-105, 2001).

[0084] Any EphA2 peptide capable of serving as an HLA-A2 restricted cytotoxic T lymphocyte (CTL) epitope may be used in accordance with the described herein. In some embodiments, the EphA2 peptide used in a vaccine described herein comprises SEQ ID NO:1. In some embodiments, the EphA2 peptide used in a vaccine described herein consists of SEQ ID NO:1.

[0085] In some embodiments, the EphA2 peptide used in accordance with the methods described herein comprises a mutated version of an EphA2 peptide, e.g., a mutated version of SEQ ID NO: 1, wherein the mutated version comprises at least 1, at least 2, or at least 3 amino acid substitutions (e.g., conservative substitutions), additions, or deletions.

[0086] In some embodiments, the EphA2 peptide used in accordance with the methods described herein comprises an amino acid sequence with at least 50%, 60%, 70%, 80%, or 90% identity to SEQ ID NO:1. In other embodiments, the EphA2 peptide used in accordance with the methods described herein comprises an amino acid sequence with at least 50% to 60%, 50% to 70%, 60% to 70%, 70% to 80%, 70% to 90%, or 80% to 90% identity to SEQ ID NO:1. In some embodiments, the EphA2 peptide used in accordance with the methods described herein comprises an amino acid sequence with at least 50%, 60%, 70%, 80%, or 90% similarity to SEQ ID NO:1. In other embodiments, the EphA2 peptide used in accordance with the methods described herein comprises an amino acid sequence with at least 50% to 60%, 50% to 70%, 60% to 70%, 70% to 80%, 70% to 90%, or 80% to 90% similarity to SEQ ID NO:1. In specific embodiments, the EphA2 peptide used in accordance with the methods described herein does not comprise or consist of SEQ ID NO: 1, i.e., the EphA2 peptide is derived from a different portion of EphA2 than is SEQ ID NO: 1.

5.3.2 Peptides derived from IL-13R α 2

[0087] IL-13R α 2 a membrane glycoprotein that binds as a component of a heterodimer to the Th2 cytokine, IL-13, which induces monocytes and macrophages to produce TGF β (see, e.g., Fichtner-Feigl et al., Nat. Med., 12: 99-106, 2006).

[0088] Any IL-13R α 2 peptide capable of serving as an HLA-A2 restricted cytotoxic T lymphocyte (CTL) epitope may be used in a vaccine described herein. In some embodiments, the IL-13R α 2 peptide used in accordance with the methods described herein comprises any one of SEQ ID NOs:2-5.

[0089] In some embodiments, the IL-13R α 2 peptide used in a vaccine described herein comprises a mutated version of SEQ ID NO:2, wherein the mutated version of SEQ ID NO:2 comprises at least 1, at least 2, or at least 3 amino acid substitutions (e.g., conservative substitutions), additions, or deletions.

[0090] In some embodiments, the IL-13R α 2 peptide used in a vaccine described herein comprises an amino acid sequence with at least 50%, 60%, 70%, 80%, or 90% identity to SEQ ID NO:2. In other embodiments, the IL-13R α 2 peptide used in a vaccine described herein comprises an amino acid sequence with at least 50% to 60%, 50% to 70%, 60% to 70%, 70% to 80%, 70% to 90%, or 80% to 90% identity to SEQ ID NO:2. In some embodiments, the IL-13R α 2 peptide used in a vaccine described herein comprises an amino acid sequence with at least 50%, 60%, 70%, 80%, or 90% similarity to SEQ ID NO:2. In other embodiments, the IL-13R α 2 peptide used in a vaccine described herein comprises an amino acid sequence with at least 50% to 60%, 50% to 70%, 60% to 70%, 70% to 80%, 70% to 90%, or 80% to 90% similarity to SEQ ID NO:2.

5.4 Immune Response Modifiers

[0091] In some embodiments, the EphA2 and/or IL-13R α 2 peptides provided herein and compositions thereof are administered concurrently with an immune response modifier. Immune response modifiers include agents capable of modifying the immune response of a subject. In some embodiments, an immune response modifier polarizes the immune response of a subject toward a Th1 response. In other embodiments, an immune response modifier polarizes the immune response of a subject toward a Th2 response. In a specific embodiment, the immune response modifier binds to a toll-like receptor (TLR) such as TLR3. Exemplary immune response modifiers that can be administered concurrently with the EphA2 and/or IL-13R α 2 peptides provided herein include, without limitation, Polyinosinic-Polycytidylic acid stabilized with polylysine and carboxymethylcellulose (poly-ICLC; also known as Hiltono1), imiquimod (Aldara®; Beselna®), and MIS-416 (Innate Therapeutics).

5.5 Adjuvants

[0092] In some embodiments, the EphA2 and/or IL-13R α 2 peptides provided herein are administered concurrently with an adjuvant. In some embodiments, the term "adjuvant" refers to an agent that when administered concurrently with or in the same composition as an EphA2 and/or IL-13R α 2 peptide augments, accelerates, prolongs, enhances and/or boosts the immune response to the IL-EphA2 and/or IL-13R α 2 peptide. In some embodiments, the adjuvant generates an immune response to the EphA2 and/or IL-13R α 2 peptide and does not produce an allergy or other adverse reaction. Adjuvants can enhance an immune response by several mechanisms including, e.g., lymphocyte recruitment, stimulation of B and/or T cells, stimulation of dendritic cells and stimulation of macrophages.

[0093] Specific examples of adjuvants include, but are not limited to, Montanide ISA-51, Montanide ISA 50V, Montanide, ISA 206, Montanide IMS 1312, VaxImmune® (CpG7909; Coley Pharmaceuticals), aluminum salts (alum) (such as aluminum hydroxide, aluminum phosphate, and aluminum sulfate), 3 De-O-acylated monophosphoryl lipid A (MPL) (see GB 2220211), MF59 (Novartis), AS03 (GlaxoSmithKline), AS04 (GlaxoSmithKline), polysorbate 80 (Tween 80; ICL Americas, Inc.), imidazopyridine compounds (see International Application No. PCT/US2007/064857, published as International Publication No. WO2007/109812), imidazoquinoxaline compounds (see International Application No. PCT/US2007/064858, published as International Publication No. WO2007/109813) and saponins, such as QS21 (see Kensil et al., in Vaccine Design: The Subunit and Adjuvant Approach (eds. Powell & Newman, Plenum Press, NY, 1995); U.S. Pat. No. 5,057,540). In some embodiments, the adjuvant is Freund's adjuvant (complete or incomplete). Other adjuvants are oil in water emulsions (such as squalene or peanut oil), optionally in combination with immune stimulants, such as monophosphoryl lipid A (see Stoute et al., N. Engl. J. Med. 336, 86-91 (1997)). Another adjuvant is CpG (Bioworld Today, Nov. 15, 1998). Such adjuvants can be used with or without other specific immunostimulating agents such as MPL or 3-DMP, QS21, polymeric or monomeric amino acids such as polyglutamic acid or polylysine, or other immunopotentiating agents. It should be understood that different formulations of EphA2 peptides may comprise different adjuvants or may comprise the same adjuvant.

5.6 Helper T Cell Epitopes

[0094] In some embodiments, the EphA2 and/or IL-13R α 2 peptides provided herein are administered concurrently with a helper T cell epitope. Helper T cell epitopes include agents that are capable of inducing a helper T cell response by the immune system. Helper T cells are CD4⁺ T cells. In some embodiments, helper T cell epitopes are presented by Class II MHC molecules, and may be recognized by the T cell receptor (TCR) of helper T cells (CD4⁺ T cells), thereby activating the CD4⁺ T cells, causing them to proliferate, secrete cytokines such as IL2, and activate professional antigen presenting cells. Through a variety of mechanisms, activated helper T cells also stimulate killer T cells (also known as CD8⁺ T cells), thereby prolonging and increasing the CD8⁺ T cell response. Exemplary helper T cell epitopes that can be administered concurrently with the EphA2 peptides provided herein include, without limitation, PADRE (see, e.g., Alexander et al, Immunity, 1:751-761, 1994), HBVcore₁₂₈₋₁₄₀, and tetanus toxoid.

5.7 Production and Purification of epha2 Peptides

[0095] The EphA2 and/or IL-13R α 2 peptides described herein can be made by standard recombinant DNA techniques or by protein synthetic techniques, e.g., by use of a peptide synthesizer. For example, a nucleic acid molecule encoding an EphA2 and/or IL-13R α 2 peptide can be synthesized by conventional techniques including automated DNA synthesizers. As another example, the EphA2 and/or IL-13R α 2 peptides described herein may be generated using conventional step-wise solution or solid phase synthesis (see, e.g., Chemical Approaches to the Synthesis of Peptides and Proteins, Williams et al., Eds., 1997, CRC Press, Boca Raton Fla., and references cited therein; Solid Phase Peptide Synthesis: A Practical Approach, Atherton & Sheppard, Eds., 1989, IRL Press, Oxford, England, and references cited therein) or through the use of segment condensation (see, e.g., Liu et al., 1996, Tetrahedron Lett. 37(7):933-936; Baca, et al., 1995, J. Am. Chem. Soc. 117:1881-1887; Tam et al., 1995, Int. J. Peptide Protein Res. 45:209-216; Schnolzer and Kent, 1992, Science 256:221-225; Liu and Tam, 1994, J. Am. Chem. Soc. 116(10):4149-4153; Liu and Tam, 1994, Proc. Natl. Acad. Sci. USA 91:6584-6588; Yamashiro and Li, 1988, Int. J. Peptide Protein Res. 31:322-334).

[0096] The EphA2 or IL-13R α 2 peptides described herein may be obtained from any information available to those of skill in the art (i.e., from Genbank, the literature, or by routine cloning). A nucleotide sequence coding for an EphA2 or IL-13R α 2 peptide can be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. A variety of host-vector systems may be utilized to express the protein-coding sequence. These include but are not limited to mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); microorganisms such as yeast (e.g. *Pichia*) containing yeast vectors; or bacteria (such as *E. coli*) transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and

specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used. In a specific embodiment, the peptide is expressed *in E. coli*. In another specific embodiment, the peptide is expressed in *Pichia*.

[0097] Once an EphA2 or IL-13R α 2 peptide has been produced by recombinant expression or by chemical synthesis, it may be purified by any method known in the art for purification of a protein, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

5.8 PHARMACEUTICAL COMPOSITIONS AND ROUTES OF ADMINISTRATION

[0098] Provided herein are pharmaceutical compositions comprising EphA2 and/or IL-13R α 2 peptides for use in the methods described herein. In certain embodiments, a composition provided herein comprises EphA2 and/or IL-13R α 2 peptide and one or more additional peptides or agents. In certain embodiments, the compositions provided herein comprise an EphA2 and/or IL-13R α 2 peptide and a helper T cell epitope, an adjuvant, and/or an immune response modifier. The pharmaceutical compositions provided herein are suitable for veterinary and/or human administration.

[0099] The pharmaceutical compositions provided herein can be in any form that allows for the composition to be administered to a subject, said subject preferably being an animal, including, but not limited to a human, mammal, or non-human animal, such as a cow, horse, sheep, pig, fowl, cat, dog, mouse, rat, rabbit, guinea pig, etc., and is more preferably a mammal, and most preferably a human.

[0100] In specific embodiments, the compositions provided herein are in the form of a liquid (e.g., an elixir, syrup, solution, emulsion, or suspension). Typical routes of administration of the liquid compositions provided herein may include, without limitation, parenteral, intradermal, intratumoral, intracerebral, and intrathecal. Parenteral administration includes, without limitation, subcutaneous, intranodal, intravenous, intramuscular, intraperitoneal, and intrapleural administration techniques. In a specific embodiment, the compositions are administered parenterally. In a composition for administration by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer, and isotonic agent may be included. In a specific embodiment, a pump may be used to deliver the vaccines (see, e.g., Sefton, CRC Crit. Ref. Biomed. Eng. 1987, 14, 201; Buchwald et al., Surgery 1980, 88: 507; Saudek et al., N. Engl. J. Med. 1989, 321: 574). In a specific embodiment, the pump may be, but is not limited to, an insulin-like pump.

[0101] Materials used in preparing the pharmaceutical compositions provided herein can be non-toxic in the amounts used. It may be evident to those of ordinary skill in the art that the optimal dosage of the active ingredient(s) in the pharmaceutical composition will depend on a variety of factors. Relevant factors include, without limitation, the type of subject (e.g., human), the overall health of the subject, the type of cancer the subject is in need of treatment of, the use of the composition as part of a multi-drug regimen, the particular form of the peptide being administered, the manner of administration, and the composition employed.

[0102] The liquid compositions provided herein, whether they are solutions, suspensions, or other like form, can also include one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which can serve as the solvent or suspending medium, polyethylene glycols, glycerin, cyclodextrin, propylene glycol, or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates, or phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose. A parenteral composition can be enclosed in an ampoule, a disposable syringe, or a multiple-dose vial made of glass, plastic or other material. An injectable composition is preferably sterile.

[0103] The compositions provided herein may comprise a pharmaceutically acceptable carrier or vehicle. As used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeiae for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the pharmaceutical composition is administered. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. The formulation should suit the mode of administration.

[0104] In one embodiment, the compositions provided herein are formulated in accordance with routine procedures as a pharmaceutical composition adapted for parenteral administration to animals, particularly human beings. Generally, the ingredients in the compositions are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachet indicating the quantity of active agent. Where a composition described herein is administered by injection, an ampoule

of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration, if necessary.

[0105] The compositions described herein can comprise an additional active agent selected from among those including, but not limited to, an additional prophylactic agent, an additional therapeutic agent, an antiemetic agent, a hematopoietic colony stimulating factor, an adjuvant therapy, an antibody/antibody fragment-based agent, an anti-depressant and an analgesic agent. In specific embodiments, the additional active agent is a second EphA2 and/or IL-13R α 2 peptide (i.e., an EphA2 and/or IL-13R α 2 peptide different from the one that forms the base of the composition). In specific embodiments, the additional active agent is a second peptide that is not an EphA2 and/or IL-13R α 2 peptide.

[0106] The pharmaceutical compositions provided herein can be prepared using methodology well known in the pharmaceutical art. For example, a composition intended to be administered by injection can be prepared by combining the EphA2 and/or IL-13R α 2 peptides described herein with water and/or other liquid components so as to form a solution. A surfactant can be added to facilitate the formation of a homogeneous solution or suspension.

[0107] The pharmaceutical compositions described herein can be included in a container, pack, or dispenser together with instructions for administration.

5.8.1 DOSAGE AND FREQUENCY OF ADMINISTRATION

[0108] The amount of a composition described herein (e.g., a composition comprising an EphA2 and/or IL-13R α 2 peptide; a composition comprising an EphA2 peptide and an IL-13R α 2 peptide a composition comprising an EphA2 and/or IL-13R α 2 peptide and a helper T cell epitope, an adjuvant) which will be effective in the treatment, prevention, and/or management of cancer may depend on the status of the cancer, the patient to whom the composition(s) is to be administered, the route of administration, and/or the type of cancer. Such doses can be determined by standard clinical techniques and may be decided according to the judgment of the practitioner.

[0109] For example, effective doses may vary depending upon means of administration, target site, physiological state of the patient (including age, body weight, health), whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human but nonhuman mammals including transgenic mammals can also be treated. Treatment dosages are optimally titrated to optimize safety and efficacy.

[0110] In certain embodiments, an *in vitro* assay is employed to help identify optimal dosage ranges. Effective doses may be extrapolated from dose response curves derived from *in vitro* or animal model test systems.

[0111] In certain embodiments, a composition comprises about 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, 600, 650, 700, 750, or 800 μ g of an EphA2 peptide per dose. In other embodiments, compositions comprise about 25 to 50, 25 to 75, 25 to 100, 50 to 100, 50 to 150, 50 to 200, 100 to 150, 100 to 200, 100 to 250, 100 to 300, 150 to 200, 150 to 250, 150 to 300, 200 to 250, 250 to 300, 250 to 350, 250 to 400, 300 to 350, 300 to 400, 300 to 450, 300 to 500, 350 to 400, 350 to 450, 400 to 500, 400 to 600, 500 to 600, 500 to 700, 600 to 700, 600 to 800, or 700 to 800 μ g of an EphA2 and/or IL-13R α 2 peptide per dose. In other embodiments, compositions comprise about 5 μ g to 100 mg, 15 μ g to 50 mg, 15 μ g to 25 mg, 15 μ g to 10 mg, 15 μ g to 5 mg, 15 μ g to 1 mg, 15 μ g to 100 μ g, 15 μ g to 75 μ g, 5 μ g to 50 μ g, 10 μ g to 50 μ g, 15 μ g to 45 μ g, 20 μ g to 40 μ g, or 25 to 35 μ g of an EphA2 and/or IL-13R α 2 peptide per kilogram of the patient.

[0112] In certain embodiments, compositions comprising an EphA2 and/or IL-13R α 2 peptide are administered concurrently with a helper T cell epitope. In some embodiments, such compositions are administered concurrently with about 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, or 600 μ g of a helper T cell epitope. In other embodiments, such compositions are administered concurrently with about 25 to 50, 25 to 75, 25 to 100, 50 to 100, 50 to 150, 50 to 200, 100 to 150, 100 to 200, 100 to 250, 100 to 300, 150 to 200, 150 to 250, 150 to 300, 200 to 250, 250 to 300, 250 to 350, 250 to 400, 300 to 350, 300 to 400, 300 to 450, 300 to 500, 350 to 400, 350 to 450, 400 to 500, 400 to 600, or 500 to 600 μ g of a helper T cell epitope.

[0113] In certain embodiments, the compositions comprising an EphA2 and/or IL-13R α 2 peptide are administered concurrently with an immune response modifier, e.g., about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, or 1800 μ g of an immune response modifier; or about 100 to 300, 200 to 400, 400 to 800, 600 to 800, 800 to 1000, 800 to 1200, 1000 to 1200, 1000 to 1400, 1200 to 1400, 1200 to 1600, 1400 to 1600, 1400 to 1800, or 1600 to 1800 μ g of an immune response modifier.

[0114] In certain embodiments, the compositions comprising an EphA2 and/or IL-13R α 2 peptide are administered concurrently with an adjuvant. In some embodiments, the compositions comprising an EphA2 peptide are mixed 0.5 to 1, 1 to 0.5, 1 to 1, 1 to 2, 1 to 3, 2 to 1, or 3 to 1 with an adjuvant.

[0115] In certain embodiments, a composition described herein is administered to a subject once as a single dose. In some embodiments, a composition described herein is administered in multiple doses (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 doses), wherein the doses may be separated by at least 1 day, 2 days, 3 days, 4, days 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 15 days, or 30 days.

[0116] In some embodiments, a composition described herein is administered over the course of 21 weeks, with administrations occurring on weeks 0, 3, 6, 9, 12, 15, 18 and 21. In certain embodiments, the composition is administered concurrently with a helper T cell epitope, an adjuvant, and/or an immune response modifier. In a specific embodiment, a composition described herein comprising is administered over the course of 21 weeks, with administrations occurring on weeks 0, 3, 6, 9, 12, 15, 18 and 21, and the composition is administered concurrently with an immune response modifier, wherein the immune response modifier is administered on the day of each administration of the composition comprising an EphA2 and/or IL-13R α 2 peptide and on day 4 after each administration of the composition comprising an EphA2 and/or IL-13R α 2 peptide. In another specific embodiment, a composition described herein is administered over the course of 21 weeks, with administrations occurring on weeks 0, 3, 6, 9, 12, 15, 18 and 21, and the composition is administered concurrently with an immune response modifier, wherein the immune response modifier is administered on the day of each administration of the composition comprising an EphA2 and/or IL-13R α 2 peptide.

5.8.2 PATIENT POPULATIONS

[0117] In certain an EphA2 and/or IL-13R α 2 peptide or composition thereof may be administered to a naïve subject, *i.e.*, a subject that does not have cancer. In one embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a naïve subject that is at risk of acquiring cancer.

[0118] In certain embodiments, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a patient who has been diagnosed with cancer. In some embodiments, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a patient with cancer before symptoms manifest or symptoms become severe. In a specific embodiment, the cancer is brain cancer.

[0119] In certain embodiments, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a patient who is in need of treatment, prevention, and/or management of cancer. Such subjects may or may not have been previously treated for cancer or may be in remission, relapsed, or may have failed treatment. Such patients may also have abnormal cytogenetics.

[0120] In a specific embodiment, the subject has been diagnosed with cancer using techniques known to one of skill in the art including, but not limited to, neurological examination; imaging methods (e.g., computed tomography (CT), magnetic resonance imaging (MRI), ultrasound, X-ray imaging, fluid-attenuated inversion-recovery (FLAIR) sequences, T2 weighted imaging, and positron emission tomography (PET) scans); and biopsy (e.g., stereotactic biopsy). Tumor response to therapy may be evaluated by McDonald criteria or Response assesment in neuro-oncology (RANO) criteria. Tumor size or response to treatment can be evaluated by various magnetic resonance imaging techniques including diffusion-weighted imaging, perfusion-weighted imaging, dynamic contrast-enhanced T1 permeability imaging, dynamic susceptibility contrast, diffusion-tensor imaging, and magnetic resonance spectroscopy, anatomic MRI T2-weighted images, fluid attenuated inversion recovery (FLAIR) T2-weighted images, and gadolinium-enhanced T1-weighted images. These imaging techniques can be used to assess tumor cellularity, white matter invasion, metabolic derangement including hypoxia and necrosis, neovascular capillary blood volume, or permeability. Positron emission tomograph (PET) technology can also be used to image tumor response, such as 18F-fluoromisonidazole PET and 3'-deoxy-3'-18F-fluorothymidine PET.

[0121] In some embodiments, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject that is in remission from brain cancer. In a specific embodiment, the subject has no detectable brain cancer, *i.e.*, no brain cancer is detectable using a conventional method described herein (e.g., MRI) or known to one of skill in the art.

[0122] In one embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with glioma. In a specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with astrocytoma (e.g., pilocytic astrocytoma, diffuse astrocytoma, and anaplastic astrocytoma). In another specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with glioblastoma. In another specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with oligodendroglioma. In another specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with brain stem glioma. In another specific embodiment, vis administered to a subject diagnosed with ependymoma. In another specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with a mixed tumor comprising more than one glial cell types.

[0123] In a specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with recurrent malignant glioma. In another specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with high-risk WHO Grade II Astrocytomas. In another specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with Oligo Astrocytoma. In another specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with recurrent WHO Grade II Glioma. In another specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with newly-diagnosed malignant

or intrinsic brain stem glioma. In another specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with incompletely resected non-brainstem glioma. In another specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with recurrent unresectable low-grade glioma.

[0124] In a specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with acoustic schwannoma. In another specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with cranial pharyngioma. In another specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with meningioma. In another specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with medulloblastoma. In another specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof described herein is administered to a subject diagnosed with primary central nervous system lymphoma. In another specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with a tumor of the pineal gland (e.g., a pineal astrocytic tumor or a pineal parenchymal tumor). In another specific embodiment, an EphA2 and/or IL-13R α 2 peptide or composition thereof is administered to a subject diagnosed with a tumor of the pituitary gland.

5.8.3 COMBINATION THERAPIES

[0125] In certain embodiments, the methods provided herein for preventing, treating, and/or managing cancer comprise administering to a patient (e.g., a human patient) in need thereof a prophylactically and/or a therapeutically effective regimen, the regimen comprising administering to the patient EphA2 and/or IL-13R α 2 peptide or composition thereof described herein and one or more additional therapies. An EphA2 peptide or composition thereof described herein and an additional therapy can be administered separately, concurrently, or sequentially. The combination therapies can act additively or synergistically. In a specific embodiment, a combination therapy provided herein comprises an EphA2 peptide and an IL-13R α 2.

[0126] The combination therapies can be administered to a subject in the same pharmaceutical composition. Alternatively, the combination therapies can be administered concurrently to a subject in separate pharmaceutical compositions. The combination therapies may be administered to a subject by the same or different routes of administration.

[0127] Any therapy (e.g., therapeutic or prophylactic agent) which is useful, has been used, or is currently being used for the prevention, treatment, and/or management of cancer (e.g., brain cancer) can be used in combination with an EphA2 and/or IL-13R α 2 peptide or composition described herein in the methods described herein. Therapies include, but are not limited to, peptides, polypeptides, antibodies, conjugates, nucleic acid molecules, small molecules, mimetic agents, synthetic drugs, inorganic molecules, and organic molecules. Non-limiting examples of cancer therapies include chemotherapy, radiation therapy, hormonal therapy, surgery, small molecule therapy, anti-angiogenic therapy, differentiation therapy, epigenetic therapy, radioimmunotherapy, targeted therapy, and/or biological therapy including immunotherapy. In certain embodiments, a prophylactically and/or therapeutically effective regimen of the invention comprises the administration of a combination of therapies.

[0128] Examples of cancer therapies which can be used in combination with EphA2 and/or IL-13R α 2 peptide or composition thereof described herein in accordance with the methods described herein include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthracyclin; anthramycin; asparaginase; asperlin; azacitidine (Vidaza); azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bisphosphonates (e.g., pamidronate (Aredia), sodium clodronate (Bonefos), zoledronic acid (Zometa), alendronate (Fosamax), etidronate, ibandronate, cimadronate, risedronate, and tiludronate); bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine (Ara-C); dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine (Dacogen); demethylation agents; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; EphA2 inhibitors; elsamitucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; flurocitabine; fosquidone; fostriecin sodium; gemcitabine; histone deacetylase inhibitors (HDACs) gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofofosine; imatinib mesylate (Gleevec, Glivec); interleukin II (including recombinant interleukin II, or rIL2), interferon alpha-2a; interferon alpha-2b; interferon alpha-n1; interferon alpha-n3; interferon beta-1a; interferon gamma-lb; iproplatin; irinotecan hydrochloride; lanreotide acetate; lenalidomide (Revlimid); letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; anti-CD2 antibodies (e.g., sipilizumab (MedImmune Inc.; International Pub-

lication No. WO 02/098370, which is incorporated herein by reference in its entirety)); megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedapa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxaliplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; pipsulfan; piroxantrone hydrochloride; plicamycin; plomes-tane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprime; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; spar-somycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblas-tine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride.

[0129] Other examples of cancer therapies which can be used in combination with an EphA2 and/or IL-13R α 2 peptide or composition thereof described herein in accordance with the methods described herein include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; al-desleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsa-crine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligo-nucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstauroporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisazirid-nylspermine; bisnafide; bistratene A; bizelesin; breffate; broprimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotria-zole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetorelix; chlorlins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentantraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol; dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadro-zole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorubicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; HMG CoA reductase inhibitors (e.g., atorvastatin, cerivastatin, fluvastatin, lescol, lupitor, lovastatin, rosuvastatin, and simvastatin); hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4- iroplact; irsogladine; isobengazole; isohomohalicondrin B; ita-setron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptol-statin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; LFA-3TIP (Biogen, Cambridge, MA; International Publication No. WO 93/0686 and U.S. Patent No. 6,162,432); liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lisso-clinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium tex-aphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocil; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mife-pristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamy-cin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; pan-omifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentozole; per-

flubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; 5-fluorouracil; leucovorin; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vaporeotide; variolin B; vector system, erythrocyte gene therapy; thalidomide; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; VITAX-IN™ (see U.S. Patent Pub. No. US 2002/0168360 A1, dated November 14, 2002, entitled "Methods of Preventing or Treating Inflammatory or Autoimmune Disorders by Administering Integrin $\alpha_v\beta_3$ Antagonists in Combination With Other Prophylactic or Therapeutic Agents"); vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

[0130] In some embodiments, the therapy(ies) used in combination with an EphA2 and/or IL-13R α_2 peptide or composition thereof described herein in accordance with the methods described herein is an immunomodulatory agent. Non-limiting examples of immunomodulatory agents include proteinaceous agents such as cytokines, peptide mimetics, and antibodies (e.g., human, humanized, chimeric, monoclonal, polyclonal, Fvs, ScFvs, Fab or F(ab)2 fragments or epitope binding fragments), nucleic acid molecules (e.g., antisense nucleic acid molecules and triple helices), small molecules, organic compounds, and inorganic compounds. In particular, immunomodulatory agents include, but are not limited to, methotrexate, leflunomide, cyclophosphamide, cytoxan, Immuran, cyclosporine A, minocycline, azathioprine, antibiotics (e.g., FK506 (tacrolimus)), methylprednisolone (MP), corticosteroids, steroids, mycophenolate mofetil, rapamycin (sirolimus), mizoribine, deoxyspergualin, brequinar, malononitriloamindes (e.g., leflunamide), T cell receptor modulators, cytokine receptor modulators, and modulators mast cell modulators. Other examples of immunomodulatory agents can be found, e.g., in U.S. Publication No. 2005/0002934 A1 at paragraphs 259-275 which is incorporated herein by reference in its entirety. In one embodiment, the immunomodulatory agent is a chemotherapeutic agent. In an alternative embodiment, the immunomodulatory agent is an immunomodulatory agent other than a chemotherapeutic agent. In some embodiments, the therapy(ies) used in accordance with the invention is not an immunomodulatory agent.

[0131] In some embodiments, the therapy(ies) used in combination with an EphA2 and/or IL-13R α_2 peptide or composition thereof described herein in accordance with the methods described herein is an anti-angiogenic agent. Non-limiting examples of anti-angiogenic agents include proteins, polypeptides, peptides, conjugates, antibodies (e.g., human, humanized, chimeric, monoclonal, polyclonal, Fvs, ScFvs, Fab fragments, F(ab)2 fragments, and antigen-binding fragments thereof) such as antibodies that specifically bind to TNF- α , nucleic acid molecules (e.g., antisense molecules or triple helices), organic molecules, inorganic molecules, and small molecules that reduce or inhibit angiogenesis. Other examples of anti-angiogenic agents can be found, e.g., in U.S. Publication No. 2005/0002934 A1 at paragraphs 277-282, which is incorporated by reference in its entirety. In a preferred embodiment, the anti-angiogenic therapy is bevacizumab (Avastin®). In other embodiments, the therapy(ies) used in accordance with the invention is not an anti-angiogenic agent.

[0132] In some embodiments, the therapy(ies) used in combination with an EphA2 and/or IL-13R α_2 peptide or composition thereof described herein in accordance with the methods described herein is an anti-inflammatory agent. Non-limiting examples of anti-inflammatory agents include any anti-inflammatory agent, including agents useful in therapies for inflammatory disorders, well-known to one of skill in the art. Non-limiting examples of anti-inflammatory agents include non-steroidal anti-inflammatory drugs (NSAIDs), steroidal anti-inflammatory drugs, anticholinergics (e.g., atropine sulfate, atropine methylnitrate, and ipratropium bromide (ATROVENT™)), beta2-agonists (e.g., albuterol (VENTOLIN™ and PROVENTIL™), bitolterol (TORNALATE™), levalbuterol (XOPONEX™), metaproterenol (ALUPENT™), pirbuterol (MAXAIR™), terbutaline (BRETHAIRE™ and BRETHINE™), albuterol (PROVENTIL™, REPETABS™, and VOLMAX™), formoterol (FORADIL AEROLIZER™), and salmeterol (SEREVENT™ and SEREVENT DISKUS™)), and methylxanthines (e.g., theophylline (UNIPHYL™, THEO-DUR™, SLO-BID™, AND TEHO-42™)). Examples of NSAIDs include, but are not limited to, aspirin, ibuprofen, celecoxib (CELEBREX™), diclofenac (VOLTAREN™), etodolac (LOD-

INE™), fenoprofen (NALFON™), indomethacin (INDOCIN™), ketoralac (TORADOL™), oxaprozin (DAYPRO™), nabumetone (RELAFEN™), sulindac (CLINORIL™), tolmentin (TOLECTIN™), rofecoxib (VIOXX™), naproxen (ALEVE™, NAPROSYN™), ketoprofen (ACTRON™) and nabumetone (RELAFEN™). Such NSAIDs function by inhibiting a cyclooxygenase enzyme (e.g., COX-1 and/or COX-2). Examples of steroidal anti-inflammatory drugs include, but are not limited to, glucocorticoids, dexamethasone (DECADRON™), corticosteroids (e.g., methylprednisolone (MEDROL™)), cortisone, hydrocortisone, prednisone (PREDNISON™ and DELTASONE™), prednisolone (PRELONE™ and PEDIAPRED™), triamcinolone, azulfidine, and inhibitors of eicosanoids (e.g., prostaglandins, thromboxanes, and leukotrienes). Other examples of anti-inflammatory agents can be found, e.g., in U.S. Publication No. 005/0002934 A1 at paragraphs 290-294, which is incorporated by reference in its entirety. In other embodiments, the therapy(ies) used in accordance with the invention is not an anti-inflammatory agent.

[0133] In certain embodiments, the therapy(ies) used in combination with an EphA2 and/or IL-13R α 2 peptide or composition thereof described herein in accordance with the methods described herein is an alkylating agent, a nitrosourea, an antimetabolite, and anthracyclin, a topoisomerase II inhibitor, or a mitotic inhibitor. Alkylating agents include, but are not limited to, busulfan, cisplatin, carboplatin, chlorambucil, cyclophosphamide, ifosfamide, decarbazine, mechlorethamine, melphalan, and temozolomide. Nitrosoureas include, but are not limited to carmustine (BCNU) and lomustine (CCNU). Antimetabolites include but are not limited to 5-fluorouracil, capecitabine, methotrexate, gemcitabine, cytarabine, and fludarabine. Anthracyclins include but are not limited to daunorubicin, doxorubicin, epirubicin, idarubicin, and mitoxantrone. Topoisomerase II inhibitors include, but are not limited to, topotecan, irinotecan, etoposide (VP-16), and teniposide. Mitotic inhibitors include, but are not limited to taxanes (paclitaxel, docetaxel), and the vinca alkaloids (vinblastine, vincristine, and vinorelbine).

[0134] Currently available cancer therapies and their dosages, routes of administration and recommended usage are known in the art and have been described in such literature as the Physician's Desk Reference (60th ed., 2006).

6. EXAMPLES

[0135] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

6.1 EXAMPLE 1

[0136] This example demonstrates that EphA2 and IL-13R α 2 are cancer stem cell antigens.

6.1.1 Materials and Methods

[0137] Flow cytometry was performed on the brain cancer cell line A-172 to assess the expression of EphA2 and IL-13R α 2 on these cancer cells. The experimental protocol included the following steps.

[0138] A-172 cells were thawed and plated in 10 cm culture dishes under sterile conditions and using aseptic technique. The A-172 cells were grown in MEM containing 10% FBS. Both cell lines were grown at 37°C with 5% CO₂ in humidified air. The A-172 cells were passaged 1:5 every 3 days.

[0139] On the day of the experiments, the cells were washed once with 1x PBS and incubated for 3 minutes with 2 ml 0.25% trypsin-EDTA at 37°C. The cells were then detached from the tissue culture plates with gentle agitation and diluted with 10 ml of DMEM. The cells then were placed in a 50 ml conical tube and centrifuged at 350 x g for 5 minutes. The supernatant was aspirated and the cells were resuspended in 10 ml DMEM. Fifty μ l of the cells were mixed with an equal volume of trypan blue and the mixture was carefully placed on a hemacytometer for counting. The cell volumes were then adjusted with DMEM to a concentration of 5x10⁶/ml.

[0140] Twenty flow cytometry tubes (Fisher Scientific) were prepared and 100 μ l of the cells were added to each tube (5 x 10⁵ cells/tube) (10 tubes with A-172 cells).

[0141] Twenty μ l of Fc blocking reagent was added to each tube and the tubes were incubated at room temperature for 10 minutes.

[0142] Ten μ l of each antibody, as provided in Table 1, below, was diluted to the described working concentration provided in Table 2, below, and was added to each appropriate tube. The tubes were incubated for 30 minutes at 4°C with gentle agitation.

TABLE 1: A-172 CELLS

Tube	#1	#2-3	#4-5	#6	#7	#8	#9	#10
Primary Antibody	Unstained	Isotype control	Secondary Antibodies Alone	α -CD133	α -IL13R α 2	α -EphA2	α -CD133 + α -IL13R α 2	α -CD133 + α -EphA2
Secondary Antibody		Anti-mouse OR Anti-goat	Anti-mouse OR Anti-goat	Anti-mouse	Anti-goat	Anti-goat	Anti-mouse + Anti-goat	Anti-mouse + Anti-goat

TABLE 2:

Antibody	Working Concentration
CD133	16.5 μ g/ml
IL13R α 2	10 μ g/ml
EphA2	50 μ g/ml
Anti-mouse-APC	1:200
Anti-goat-FITC	1:200

[0143] After the incubation, the cells were centrifuged at 300 x g for 1 minute in a tabletop, refrigerated microcentrifuge. The supernatant was removed and the cells were washed with ice cold FACS buffer 3 times. The cells were then resuspended in 100 μ l of FACS buffer and 10 μ l of the secondary antibodies was added to the appropriate tubes. The tubes were incubated for 30 minutes at 4°C with gentle agitation in the dark.

[0144] After the incubation, the cells were centrifuged at 300 x g for 1 minute in a tabletop, refrigerated microcentrifuge. The supernatant was removed and the cells were washed with ice cold FACS buffer 3 times. The cells were then resuspended in 200 μ l of FACS buffer and analyzed on a FACSCalibur (BD Biosciences) flow cytometer.

6.1.2 Results

[0145] In brain cancer, the brain cancer stem cells can be identified using the marker CD133, i.e., brain cancer stem cells are known to express the CD133 antigen (see, e.g., Singh et al., 2004, Nature 432:396-401, the disclosure of which is hereby incorporated by reference in its entirety). The cancer stem cells of the brain cancer cell line A-172 express CD133 (see, e.g., Qiang et al., 2009, Cancer Letters 271:13-21, the disclosures of which is hereby incorporated by reference in its entirety).

[0146] As demonstrated in Figure 1, all cells of the A-172 line were positive for EphA2 and IL-13R α 2, whereas a small population of such cells also were positive for CD133. This CD133+ cell subpopulation thus represents the cancer stem cell subpopulation of the A-172 cell line, and the same expression pattern of CD133 on A-172 cells was observed in a subsequent duplicate experiment (see Figures 6 and 7).

[0147] As demonstrated in Figure 2, the CD133+ population also was positive for expression of EphA2, thus demonstrating that EphA2 is present on the cancer stem cell population obtained from the A-172 cell line, and thus that EphA2 is a cancer stem cell antigen. This fact was verified in a subsequent duplicate experiment (see Figure 8). Moreover, as shown in Figure 4, EphA2 was expressed to higher levels on CD133+ Cells as compared to CD133- cells

[0148] Similarly, as demonstrated by Figure 2, the CD133+ population of A-172 cell line also was positive for expression of IL-13R α 2, thus demonstrating that IL-13R α 2 is present on the cancer stem cell population obtained from the A-172 cell line, and thus that IL-13R α 2 is a cancer stem cell antigen. This fact was verified in a subsequent duplicate experiment (see Figure 8). Moreover, as shown in Figure 5, IL-13R α 2 is was expressed to higher levels on CD133+ Cells as compared to CD133- cells.

6.1.3 Conclusion

[0149] These data demonstrate that EphA2 is a cancer stem antigen, and thus can be used in methods for the treatment of cancer, such as brain cancer.

Equivalents:

[0150] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

[0151] Various publications, patents and patent applications are cited herein, the disclosures of which are incorporated by reference in their entireties.

[0152] Embodiments of the invention may include the features of the following enumerated paragraphs ("paras").

1. A method for treating, preventing, or managing cancer in a subject in need thereof comprising (i) administering to said subject a composition comprising an EphA2 peptide and (ii) measuring the amount of cancer stem cells in said subject.

2. The method of para 1, wherein the amount of cancer stem cells in said subject is reduced and or does not increase.

3. A method for monitoring the efficacy of an EphA2 peptide-based cancer therapy in a patient with cancer, the method comprising: (a) measuring the amount of cancer stem cells in or from the patient before and following the administration of the cancer therapy; and (b) comparing the amount of cancer stem cells in or from the patient before the administration of the cancer therapy to the amount of cancer stem cells in or from the patient following the administration of the cancer therapy; wherein the cancer therapy is determined to be efficacious if the amount cancer stem cells in or from the patient following the administration of the cancer therapy is equivalent to or less than the amount of cancer stem cells in or from the patient before the administration of the cancer therapy.

4. The method of para 1 or 3, wherein the amount of cancer stem cells is determined using a biological fluid, a bone marrow biopsy, a tumor biopsy, or a normal tissue biopsy from the patient.

5. The method of para 1 or 3, wherein the amount of cancer stem cells is determined by using an immunoassay.

6. The method of para 5, wherein the immunoassay is selected from the group consisting of western blots, immunohistochemistry, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, immunofluorescence, protein A immunoassays, flow cytometry and FACS analysis.

7. The method of para 1 or 3, wherein the amount of cancer stem cells is determined using a flow cytometer.

8. The method of para 7, wherein the amount of cancer stem cells is determined with one or more antibodies that bind cell surface markers.

9. The method of para 7, wherein the cancer stem cells are contacted with one or more dyes prior to detection in the flow cytometer.

10. The method of para. 1 or 3, wherein the amount of cancer stem cells is determined by immunohistochemistry.

11. The method of para 1 or 3, wherein the amount of cancer stem cells is determined using a sphere forming assay.

12. The method of para 1 or 3, wherein the amount of cancer stem cells is determined by culturing a sample obtained from the patient, or a portion thereof, and quantitating the cells in an *in vitro* assay.

13. The method of para 1 or 3, wherein the amount of cancer stem cells is determined by using an immunocompromised mouse *in vivo* engraftment assay.

14. The method of para 1 or 3, wherein the amount of cancer stem cells is determined using imaging.

15. The method of para 14, wherein said imaging MRI, PET, FDG-PET, CT scan, or X-RAY.

16. The method of para 1, further comprising comparing the amount of cancer stem cells in a sample obtained from the patient to the amount of cancer stem cells in a reference sample, or to a predetermined reference range, wherein

a stabilization or a decrease in the amount of cancer stem cells in the sample relative to the reference sample, or to a predetermined reference range, indicates that the method is effective.

17. The method of any one of paras 1 to 17, wherein said cancer stem cells are associated with a brain cancer tumor.

18. The method of para. 2, wherein said amount is measured using a biopsy from the patient.

19. The method of para 18, wherein said amount is measured by subjecting the cells of said biopsy to flow cytometry, immunohistochemistry, neurosphere formation, or growth in an immunodeficient mouse.

20. The method of para 1, wherein said EphA2 peptide is a T cell epitope of EphA2.

21. The method of para . 20, wherein said T cell epitope of EphA2 induces an immune response in a subject.

22. The method of para 1 or 20, wherein said EphA2 peptide comprises SEQ ID NO.:1.

23. The method of para 1 or 20, wherein said EphA2 peptide consists of SEQ ID NO.:1.

24. The method of para 1, wherein said composition is cell free.

25. The method of para 1, wherein said composition comprises cells.

26. The method of para 1, wherein said EphA2 peptide in said composition is loaded on dendritic cells.

27. The method of para 1, wherein said composition is administered to the subject subcutaneously or intra-nodally.

28. A method of treating cancer in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a compound that targets EphA2.

29. The method of para. 28, wherein said compound is an antibody that binds to EphA2.

30. The method of para 29, wherein said antibody is a monoclonal antibody, a single chain antibody, a bispecific antibody, or a fragment of an EphA2-specific antibody.

31. The method of para . 29, wherein said cancer is brain cancer.

32. The method of para 29, wherein said antibody binds EphA2-expressing cancer stem cells.

33. The method of para 28, wherein said method further comprises measuring the amount of cancer stem cells in said subject.

34. The method of para 33, wherein said measuring is performed before, during, and after administration of said compound.

35. The method of para 33 or 34, wherein the amount of cancer stem cells is determined using a biological fluid, a bone marrow biopsy, a tumor biopsy, or a normal tissue biopsy from the patient.

36. The method of para 33 or 34, wherein the amount of cancer stem cells is determined by using an immunoassay.

37. The method of para 36, wherein the immunoassay is selected from the group consisting of western blots, immunohistochemistry, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, immunofluorescence, protein A immunoassays, flow cytometry and FACS analysis.

38. The method of para 33 or 34, wherein the amount of cancer stem cells is determined using a flow cytometer.

39. The method of para 38, wherein the amount of cancer stem cells is determined with one or more antibodies that

bind cell surface markers.

40. The method of para 38, wherein the cancer stem cells are contacted with one or more dyes prior to detection in the flow cytometer.

41. The method of para 33 or 34, wherein the amount of cancer stem cells is determined by immunohistochemistry.

42. The method of para 33 or 34, wherein the amount of cancer stem cells is determined using a sphere forming assay.

43. The method of para. 33 or 34, wherein the amount of cancer stem cells is determined by culturing a sample obtained from the patient, or a portion thereof, and quantitating the cells in an *in vitro* assay.

44. The method of para 33 or 34, wherein the amount of cancer stem cells is determined by using an immunocompromised mouse *in vivo* engraftment assay.

45. The method of para 33 or 34, wherein the amount of cancer stem cells is determined using imaging.

46. The method of para 45, wherein said imaging MRI, PET, FDG-PET, CT scan, or X-RAY.

47. A method of improving the targeting of cancer stem cells with a cancer vaccine comprising determining the binding motif of a Class I or Class II epitope from EphA2, and making substitutions in the amino acid sequence such that the modified peptides are able to induce an immune response that is at least as effective at killing cancer stem cells as the wild type peptide.

48. The method of para 47, wherein the substitutions are in at least one amino acid involved in binding to the MHC molecule.

49. The method of para 47, wherein the substitutions are in at least one amino acid involved in contacting the T cell receptor.

50. The method of para 47, wherein the substitutions are in at least one amino acid involved in altering the conformation of the peptide such the substitution affects either the binding the MHC molecule or the contacting of the T cell receptor.

51. A method for treating, preventing, or managing cancer in a subject in need thereof comprising (i) administering to said subject a cancer vaccine, wherein said cancer vaccine comprises an EphA2 peptide and (ii) measuring the amount of cancer stem cells in said subject.

52. The method of para 1, 3, or 51 wherein the amount of cancer stem cells is measured by determining the amount of EphA2-expressing cancer stem cells, IL-13R α 2-expressing cancer stem cells, or CD133-expressing cancer stem cells.

53. A method for treating, preventing, or managing cancer in a subject in need thereof comprising (i) administering to said subject a composition comprising an IL-13R α 2 peptide and (ii) measuring the amount of cancer stem cells in said subject.

54. The method of para 1, wherein the amount of cancer stem cells in said subject is reduced and or does not increase.

55. A method for monitoring the efficacy of an IL-13R α 2 peptide-based cancer therapy in a patient with cancer, the method comprising: (a) measuring the amount of cancer stem cells in or from the patient before and following the administration of the cancer therapy; and (b) comparing the amount of cancer stem cells in or from the patient before the administration of the cancer therapy to the amount of cancer stem cells in or from the patient following the administration of the cancer therapy; wherein the cancer therapy is determined to be efficacious if the amount cancer stem cells in or from the patient following the administration of the cancer therapy is equivalent to or less than the amount of cancer stem cells in or from the patient before the administration of the cancer therapy.

56. The method of para 53, further comprising comparing the amount of cancer stem cells in a sample obtained from the patient to the amount of cancer stem cells in a reference sample, or to a predetermined reference range,

wherein a stabilization or a decrease in the amount of cancer stem cells in the sample relative to the reference sample, or to a predetermined reference range, indicates that the method is effective.

5 57. A method of treating cancer in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a compound that targets IL-13R α 2.

58. The method of para 28, wherein said compound is an antibody that binds to IL-13R α 2.

10 59. A method of improving the targeting of cancer stem cells with a cancer vaccine comprising determining the binding motif of a Class I or Class II epitope from IL-13R α 2, and making substitutions in the amino acid sequence such that the modified peptides are able to induce an immune response that is at least as effective at killing cancer stem cells as the wild type peptide.

15 60. A method for treating, preventing, or managing cancer in a subject in need thereof comprising (i) administering to said subject a cancer vaccine, wherein said cancer vaccine comprises an IL-13R α 2 peptide and (ii) measuring the amount of cancer stem cells in said subject.

20 61. The method of para 53, 55, or 60, wherein the amount of cancer stem cells is measured by determining the amount of EphA2-expressing cancer stem cells, IL-13R α 2-expressing cancer stem cells, or CD133-expressing cancer stem cells.

SEQUENCE LISTING

<110> STEMLINE THERAPEUTICS, INC.

<120> METHODS FOR TREATING AND MONITORING THE
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<130> P15442EP01

<140> To be assigned

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Claims

1. A composition comprising an IL-13R α 2 peptide and an EphA2 peptide for use in a method of treating, preventing, or managing cancer in a subject in need thereof comprising
 - (i) administering said composition to said subject and
 - (ii) measuring the amount of cancer stem cells in said subject.
2. The composition for use according to claim 1, wherein the method further comprises
 - (iii) comparing the amount of cancer stem cells in a sample obtained from the patient to the amount of cancer stem cells in a reference sample, or to a predetermined reference range, wherein a stabilization or a decrease in the amount of cancer stem cells in the sample relative to the reference sample, or to a predetermined reference range, indicates that the method is effective.
3. The composition for use according to claim 1 or 2, wherein said IL-13R α 2 peptide and said EphA2 peptide are loaded on dendritic cells.
4. The composition for use according to claim 1 or 2, wherein:
 - (a) said IL-13R α 2:
 - (i) is a T cell epitope of IL-13R α 2, preferably wherein said T cell epitope of IL-13R α 2 induces an immune response in a subject; or
 - (ii) comprises any one of SEQ ID NOs: 2, 3, 4, or 5, or consists of any one of SEQ ID NOs: 2, 3, 4, or 5;
 - and/or
 - (b) said EphA2:
 - (i) is a T cell epitope of EphA2, preferably wherein said T cell epitope of EphA2 induces an immune response in a subject; or
 - (ii) comprises SEQ ID NOs: 1, or consists of SEQ ID NO: 1.
5. A method for monitoring the efficacy of an IL-13R α 2 and EphA2 peptide-based cancer therapy in a patient with cancer, the method comprising:

(a) measuring the amount of cancer stem cells taken from the patient before and following the administration of the IL-13R α 2 and EphA2 peptide-based cancer therapy; and

(b) comparing the amount of cancer stem cells taken from the patient before the administration of the cancer therapy to the amount of cancer stem cells taken from the patient following the administration of the IL-13R α 2 and EphA2 peptide-based cancer therapy, wherein the cancer therapy is determined to be efficacious if the amount cancer stem cells taken from the patient following the administration of the cancer therapy is equivalent to or less than the amount of cancer stem cells taken from the patient before the administration of the cancer therapy.

6. The composition for use according to claims 1 to 4 or the method according to claim 5, wherein said amount of cancer stem cells is measured by using a biopsy, a biological fluid, a bone marrow biopsy, a tumor biopsy, or a normal tissue biopsy from the subject or patient, respectively.

7. The composition for use according to claims 1 to 4 or the method according to claim 5, wherein said amount of cancer stem cells is measured by determining the amount of IL-13R α 2-expressing cancer stem cells and/or EphA2-expressing cancer stem cells, or CD 133-expressing cancer stem cells.

8. The composition for use according to claims 1 to 4 or the method according to claim 5, wherein said amount of cancer stem cells is measured by:

(i) using an immunoassay, wherein the immunoassay is chosen from western blots, immunohistochemistry, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, immunofluorescence, protein A immunoassays, flow cytometry and FACS analysis;

(ii) using a flow cytometer, wherein the amount of cancer stem cells is preferably determined with one or more antibodies that bind cell surface markers, or wherein the cancer stem cells are preferably contacted with one or more dyes prior to detection in the flow cytometer;

(iii) immunohistochemistry;

(iv) using a sphere forming assay;

(v) culturing a sample obtained from the subject, or a portion thereof, and quantitating the cells in an in vitro assay;

(vi) using an immunocompromised mouse in vivo engraftment assay; or

(vii) using imaging, wherein said imaging is preferably MRI, PET, FDG-PET, CT scan, or X-RAY.

9. The composition for use according to claims 1 to 4 or the method according to claim 5, wherein said cancer stem cells are associated with a brain cancer.

10. The composition for use or the method according to claim 9, wherein said brain cancer is glioma, glioblastoma, oligodendroglioma, brain stem glioma, non-brain stem glioma, ependymoma, acoustic schwannoma, cranialpharyngioma, meningioma, medulloblastoma, primary central nervous system lymphoma, tumors of the pineal and pituitary glands, adult low-grade infiltrative supratentorial astrocytoma/oligodendroglioma, adult low-grade infiltrative supratentorial astrocytoma, adult low-grade infiltrative supratentorial oligodendroglioma, adult low-grade infiltrative supratentorial astrocytoma/oligodendroglioma (excluding pilocytic astrocytoma), adult low-grade infiltrative supratentorial astrocytoma (excluding pilocytic astrocytoma), adult low-grade infiltrative supratentorial oligodendroglioma (excluding pilocytic astrocytoma), adult intracranial ependymoma, adult intracranial ependymoma (excluding subependymoma and myxopapillary), adult intracranial anaplastic ependymoma, anaplastic glioma, anaplastic glioblastoma, pilocytic astrocytoma, subependymoma, myxopapillary, leptomeningeal metastases, primary CNS lymphoma, metastatic spine tumor, or meningioma.

11. The composition for use or the method according to claim 10, wherein said glioma is astrocytoma, pilocytic astrocytoma, diffuse astrocytoma, anaplastic astrocytoma, high risk WHO Grade II astrocytoma, oligo astrocytoma, recurrent malignant glioma, recurrent WHO Grade II glioma, newly-diagnosed malignant or intrinsic brain stem glioma, incompletely resected non-brainstem glioma, or recurrent unresectable low-grade glioma.

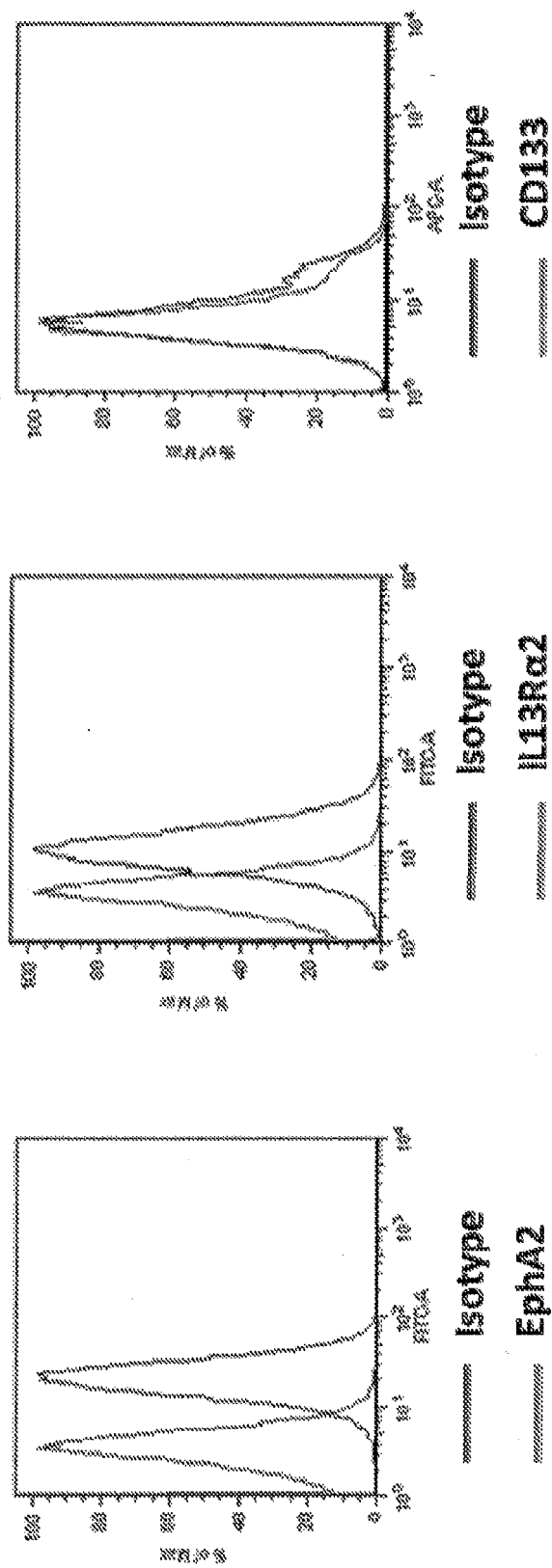


FIG. 1

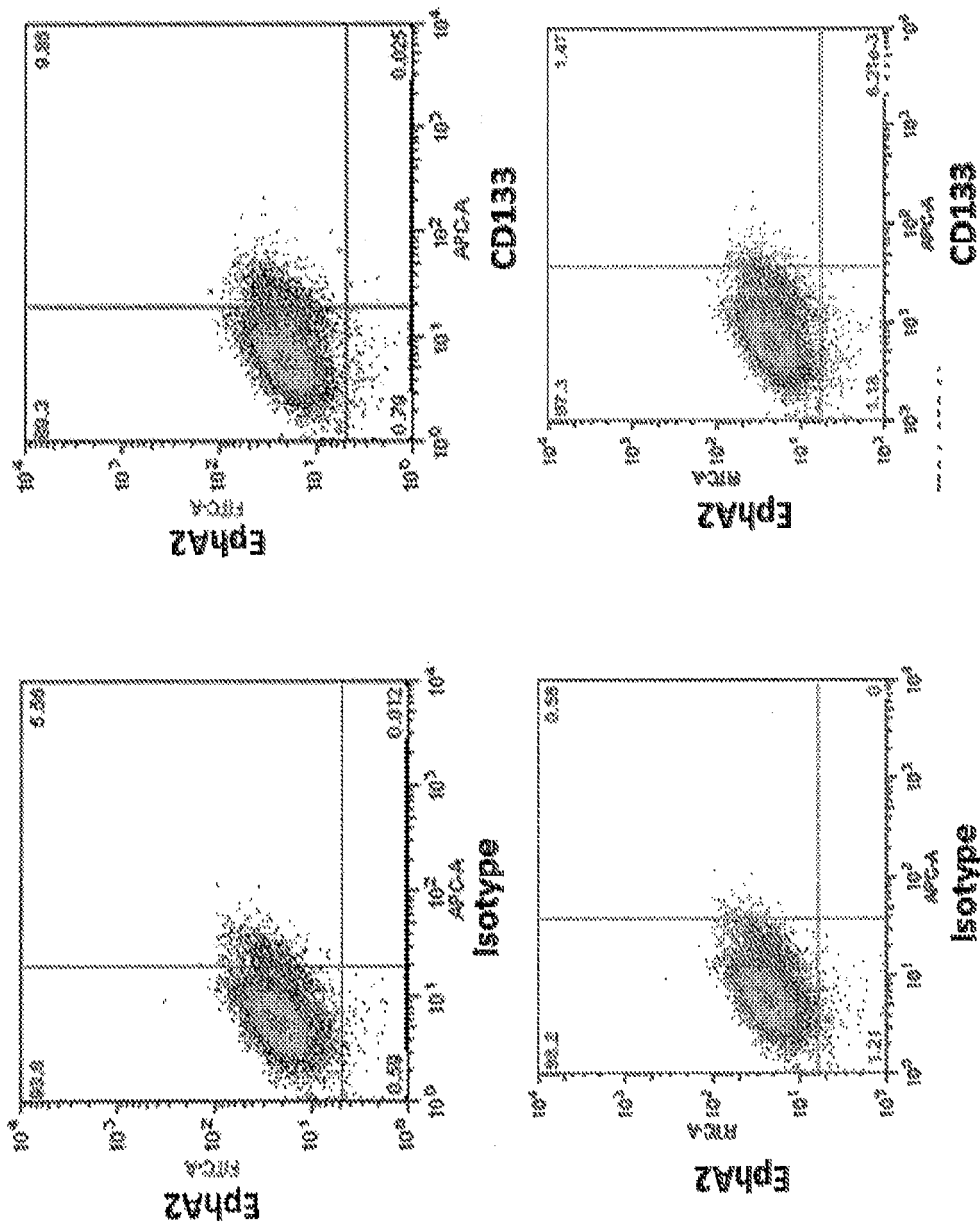


FIG. 2

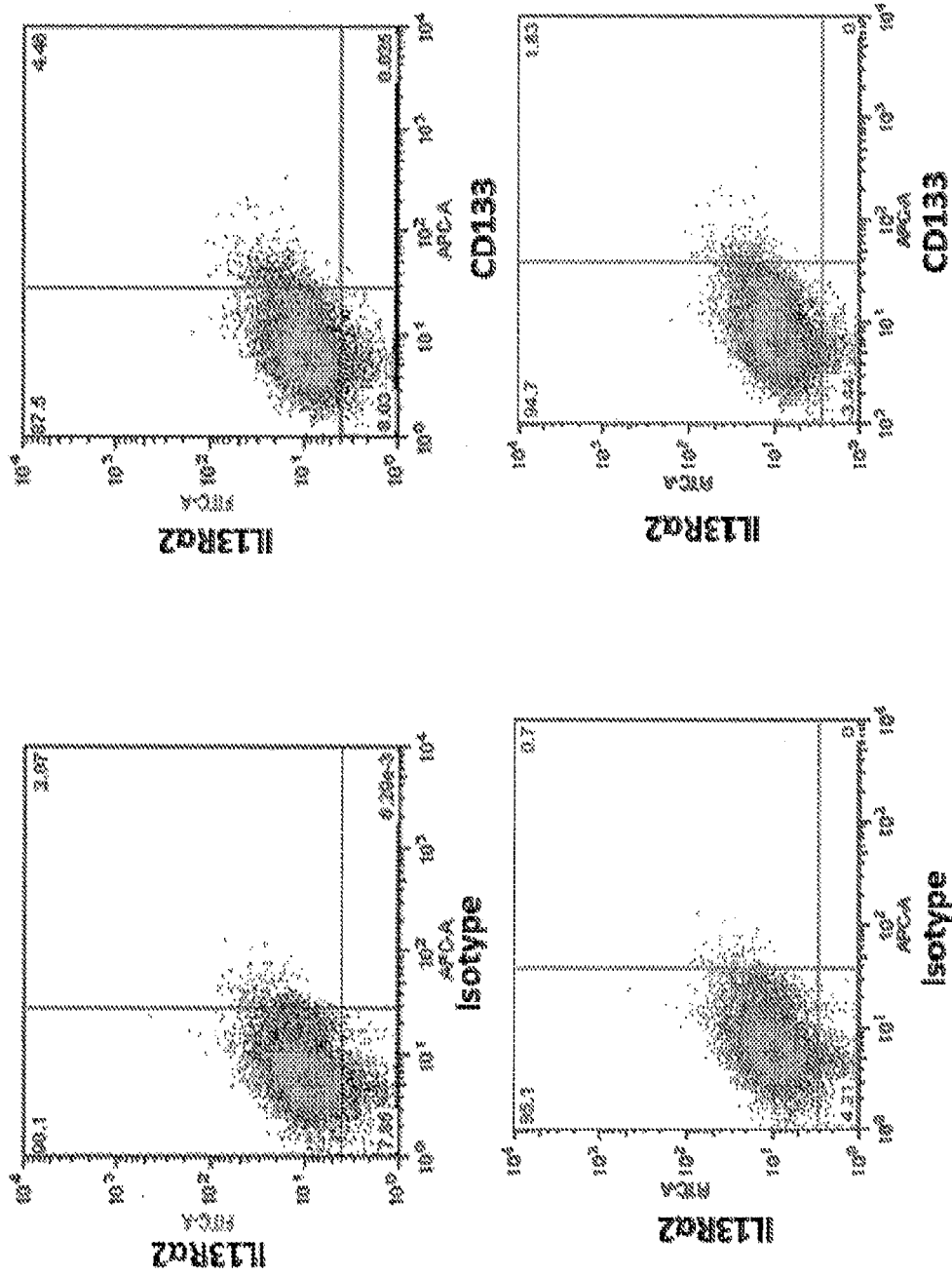


FIG. 3

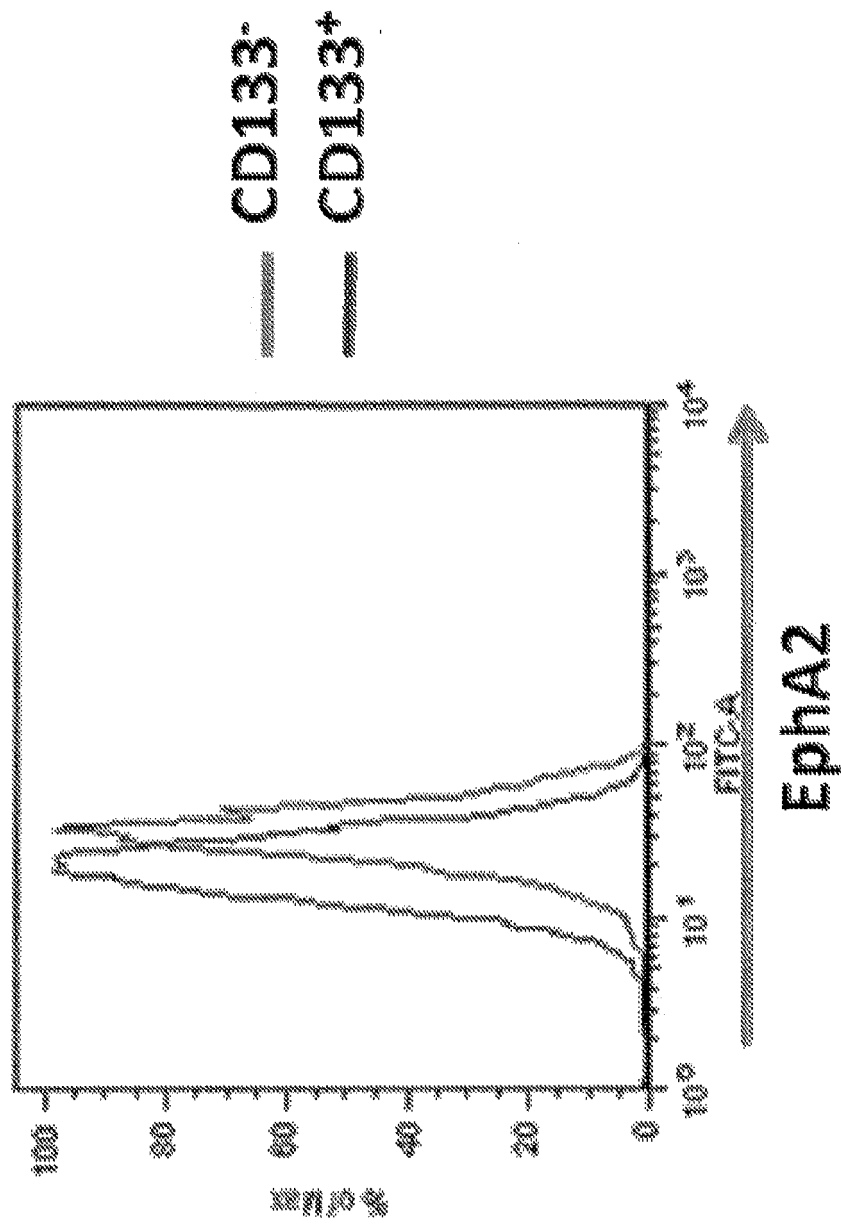


FIG. 4

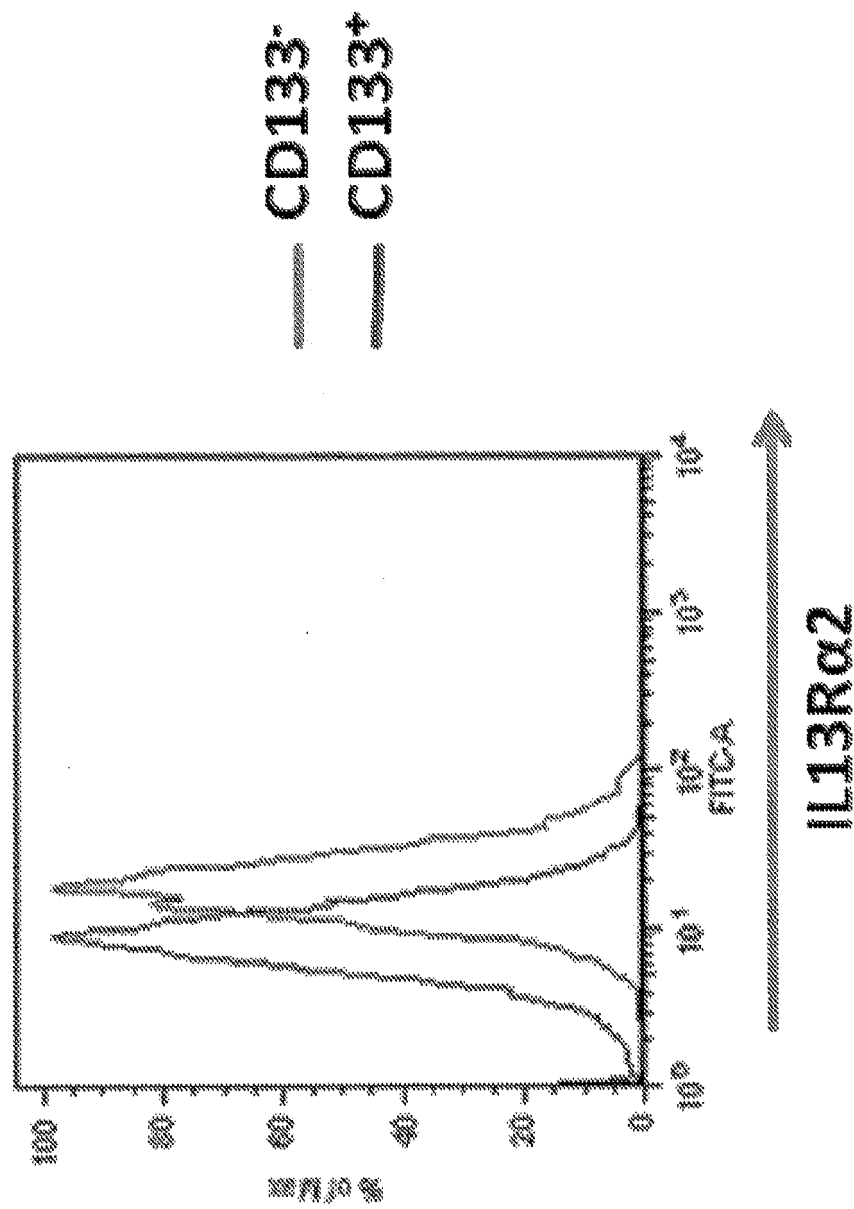


FIG. 5

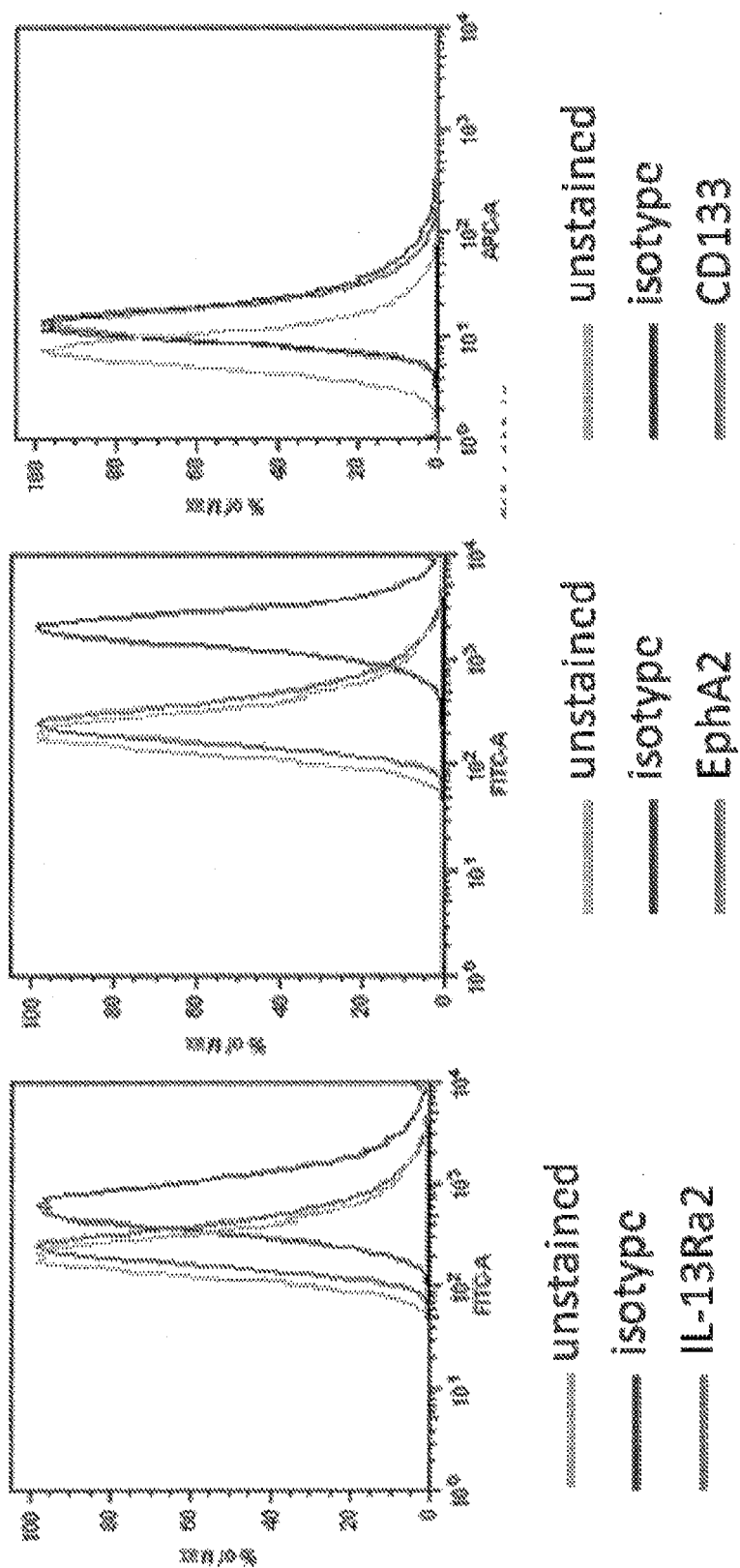


FIG. 6

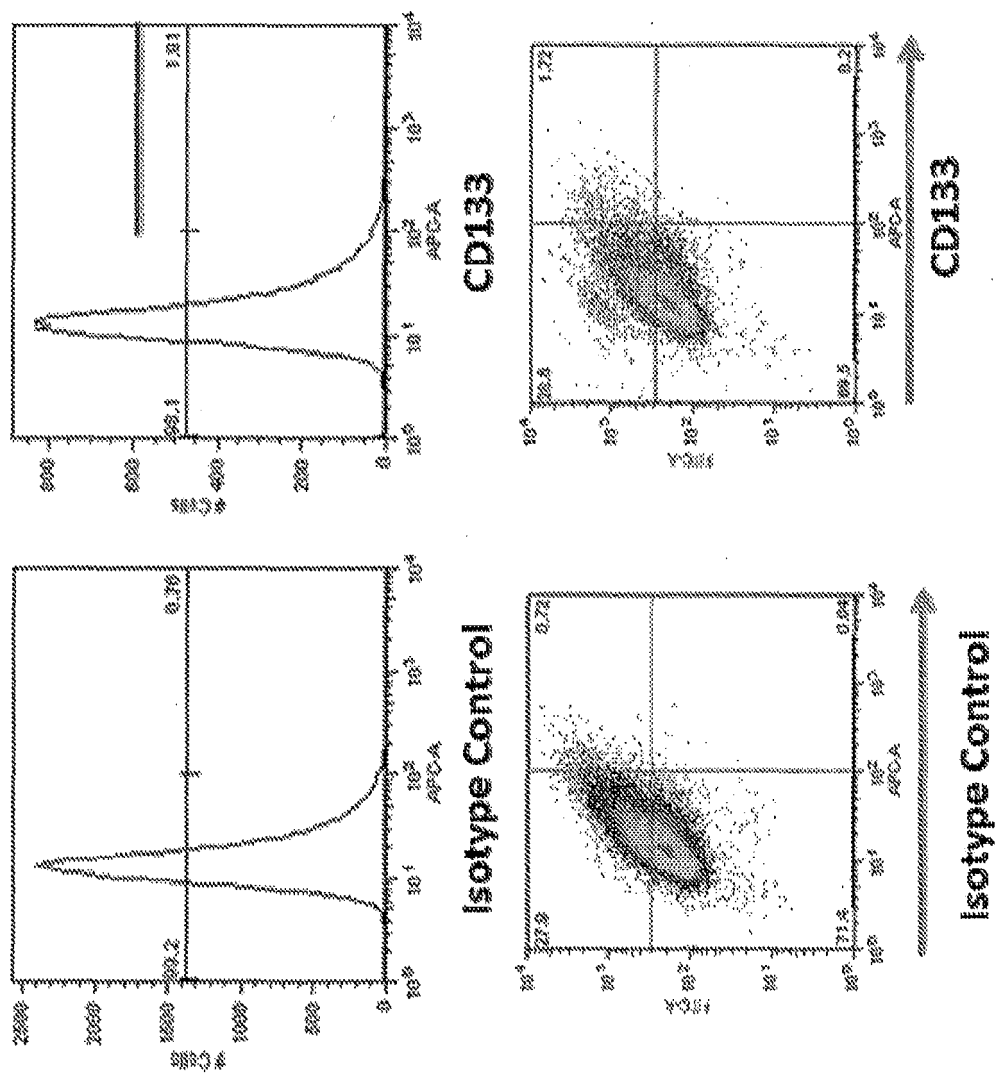


FIG. 7

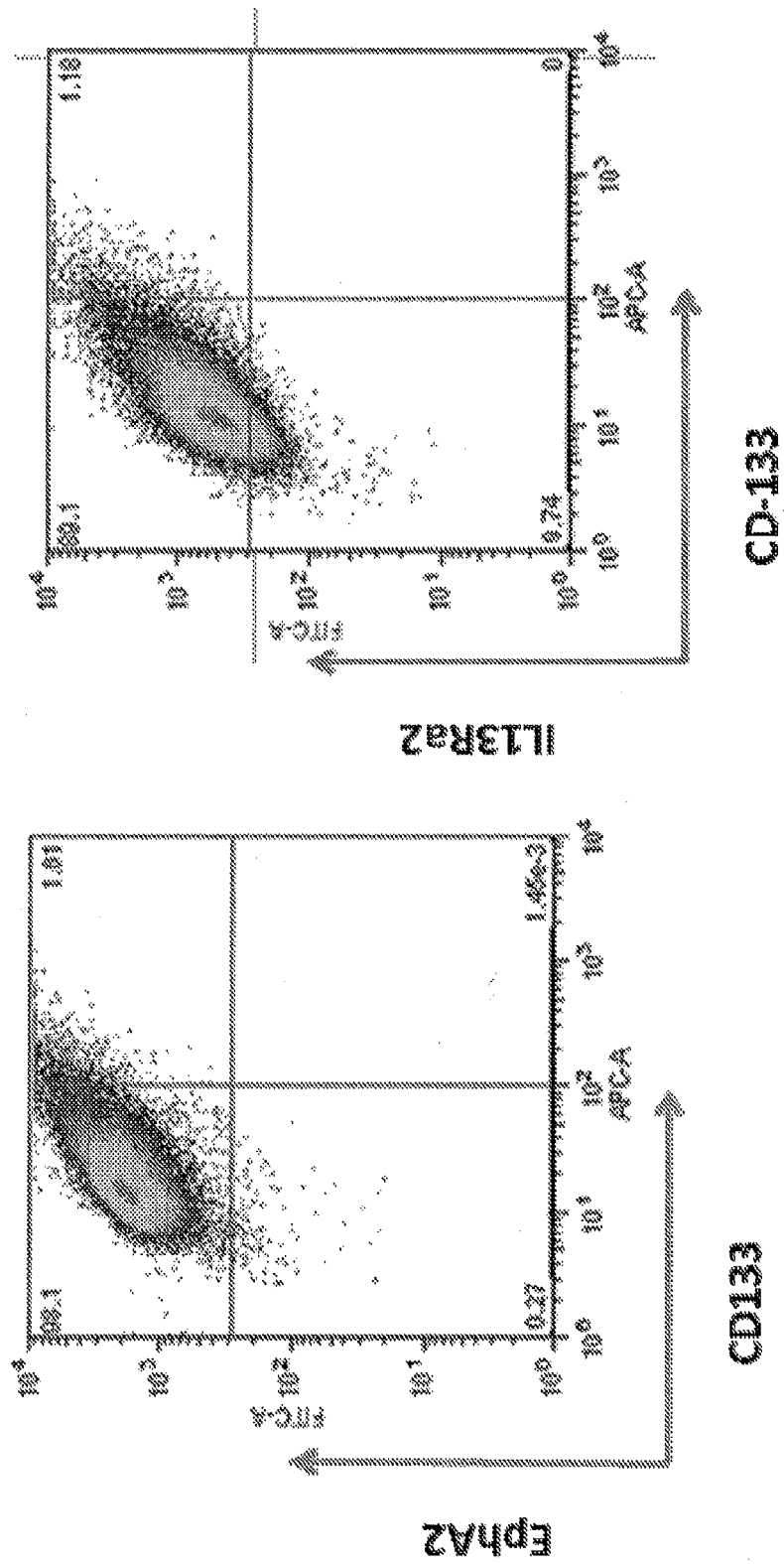


FIG. 8



EUROPEAN SEARCH REPORT

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	BRIAN J MORRISON ET AL: "Breast cancer stem cells: implications for therapy of breast cancer", BREAST CANCER RESEARCH, vol. 10, no. 4, 22 July 2008 (2008-07-22), pages 1-14, XP055218654, GB ISSN: 1465-5411, DOI: 10.1186/bcr2111 * the whole document * * In particular: P. 8-11, Section with title: Immunotherapy targeting breast cancer stem cells; More particularly p. 9-10 bridging paragraph and p. 10-11, bridging paragraph. * -----	1-11	INV. G01N33/574
A	M. DE LA LUZ GARCIA-HERNANDEZ ET AL: "Prostate Stem Cell Antigen Vaccination Induces a Long-term Protective Immune Response against Prostate Cancer in the Absence of Autoimmunity", CANCER RESEARCH, vol. 68, no. 3, 1 February 2008 (2008-02-01), pages 861-869, XP055218655, US ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-07-0445 * the whole document * * In particular: Title; Abstract; Materials and methods section, p. 862, 2nd column, 3rd and 4th paragraph. * ----- -/--	1-11	TECHNICAL FIELDS SEARCHED (IPC) G01N A61K C07K C12N
The present search report has been drawn up for all claims			
Place of search The Hague		Date of completion of the search 7 May 2021	Examiner C.F. Angioni
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

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A	MADHAV V. DHODAPKAR ET AL: "Vaccines Targeting Cancer Stem Cells", THE CANCER JOURNAL, vol. 17, no. 5, 1 September 2011 (2011-09-01), pages 397-402, XP055218375, ISSN: 1528-9117, DOI: 10.1097/PP0.0b013e318233e730 * the whole document * * In particular: p. 2-3, bridging paragraph; p. 4-5, bridging paragraph; p. 5-6, bridging paragraph. *	1-11	TECHNICAL FIELDS SEARCHED (IPC)
A	HATANO M ET AL: "EPHA2 AS A GLIOMA-ASSOCIATED ANTIGEN: A NOVEL TARGET FOR GLIOMA VACCINES", NEOPLASIA, NEOPLASIA PRESS, ANN ARBOR, MI, US, vol. 7, no. 8, 1 August 2005 (2005-08-01), pages 717-722, XP008076099, ISSN: 1522-8002, DOI: 10.1593/NEO.05277 * the whole document *	1-11	
A	US 2008/175870 A1 (MATHER JENNIE P [US] ET AL) 24 July 2008 (2008-07-24) * the whole document * * In particular: paragraphs 53, 54 and 116-130. *	1-11	
A	US 2011/229504 A1 (FRITSCH JENS [DE] ET AL) 22 September 2011 (2011-09-22) * the whole document * * In particular: Paragraphs 82, 97, 212-215, 250, 265; claims 1-28. *	1-11	
The present search report has been drawn up for all claims			
Place of search The Hague		Date of completion of the search 7 May 2021	Examiner C.F. Angioni
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			



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A	TOSHIO FUJISAWA ET AL: "Targeting IL-13R[alpha]2 in human pancreatic ductal adenocarcinoma with combination therapy of IL-13-PE and gemcitabine", INTERNATIONAL JOURNAL OF CANCER, vol. 128, no. 5, 1 March 2011 (2011-03-01), pages 1221-1231, XP055236401, US ISSN: 0020-7136, DOI: 10.1002/ijc.25437 * the whole document * * In particular: Title; abstract; Materials and Methods section; Figures 1-5; Table 1. *	1-11	
A	VAN NGUYEN ET AL: "IL-13R[alpha]2-Targeted Therapy Escapees: Biologic and Therapeutic Implications", TRANSLATIONAL ONCOLOGY, vol. 4, no. 6, 1 December 2011 (2011-12-01), pages 390-400, XP055236405, United States ISSN: 1936-5233, DOI: 10.1593/tlo.11175 * the whole document * * In particular: Title; Abstract; Materials and methods section; Figures 1-6. *	1-11	
The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (IPC)
Place of search The Hague		Date of completion of the search 7 May 2021	Examiner C.F. Angioni
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	C. E. BROWN ET AL: "Stem-like Tumor-Initiating Cells Isolated from IL13R 2 Expressing Gliomas Are Targeted and Killed by IL13-Zetakine-Redirected T Cells", CLINICAL CANCER RESEARCH, vol. 18, no. 8, 8 March 2012 (2012-03-08), pages 2199-2209, XP055218517, US ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-11-1669 * the whole document * * In particular: Title; Abstract; Materials and methods section; p. 6, last paragraph - p. 7, 3rd paragraph; Figures 1-6. *	1-11	
A	US 2012/052080 A1 (OKADA HIDEHO [US]) 1 March 2012 (2012-03-01) * the whole document * * In particular: Paragraphs 227-714, examples 1-10; Claims 1-45. *	1-11	TECHNICAL FIELDS SEARCHED (IPC)
The present search report has been drawn up for all claims			
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CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

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**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 20 21 1725

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
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